CANADIAN JOURNAL OF RESEARCH

VOLUME 24 OCTOBER, 1946 NUMBER 5

— SECTION C —

BOTANICAL SCIENCES

Contents

	Page
Inheritance of Variegation in Barley—*T. J. Arnason, J. B. Harrington, and H. A. Friesen*	145
Investigations of Decay in Western Red Cedar in British Columbia—*D. C. Buckland*	158
On Staining for Vitamin C in Tissues—*Jules Tuba, George Hunter, and John A. Osborne*	182
Cultural and Taxonomic Studies on Certain Fusaria. I. Mutation in Culture—*J. J. Miller*	188
Cultural and Taxonomic Studies on Certain Fusaria. II. The Taxonomic Problem in *Fusarium* with Particular Reference to Section Elegans—*J. J. Miller*	213
The Microflora of Stored Wheat—*Norman James, Joyce Wilson, and Egon Stark*	224

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

CANADIAN JOURNAL OF RESEARCH

The *Canadian Journal of Research* is issued in six sections, as follows:

A. Physical Sciences
B. Chemical Sciences
C. Botanical Sciences
D. Zoological Sciences
E. Medical Sciences
F. Technology

For the present, each of these sections is to be issued six times annually, under separate cover, with separate pagination.

The *Canadian Journal of Research* is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. The *Canadian Journal of Research* is edited by a joint Editorial Board consisting of members of the National Research Council of Canada, the Royal Society of Canada, and the Chemical Institute of Canada.

Sections B and F of the *Canadian Journal of Research* have been chosen by the Chemical Institute of Canada as its medium of publication for scientific papers.

EDITORIAL BOARD

Representing
NATIONAL RESEARCH COUNCIL

DR. R. NEWTON (*Chairman*)
President,
University of Alberta, Edmonton.

DR. J. B. COLLIP,
Director, Research Institute
of Endocrinology,
McGill University, Montreal.

DR. J. A. GRAY,
Professor of Physics,
Queen's University, Kingston.

DR. O. MAASS,
Professor of Physical Chemistry,
McGill University, Montreal.

Representing
ROYAL SOCIETY OF CANADA

DR. C. C. COFFIN,
Professor of Chemistry,
Dalhousie University, Halifax.

PROF. J. K. ROBERTSON,
Department of Physics,
Queen's University, Kingston.

Section III

PROF. J. R. DYMOND,
Royal Ontario Museum of
Zoology, Toronto.

DR. H. S. JACKSON,
Professor of Botany,
University of Toronto, Toronto.

Section V

Ex officio

DR. W. H. COOK, Editor-in-Chief,
Director, Division of Applied Biology,
National Research Laboratories, Ottawa.

CHEMICAL INSTITUTE OF CANADA

DR. R. V. V. NICHOLLS,
Assistant Professor of Chemistry,
McGill University, Montreal.

EDITORIAL COMMITTEE

Editor-in-Chief, DR. W. H. COOK
Editor Section A, PROF. J. K. ROBERTSON
Editor Section B, DR. C. C. COFFIN
Editor Section C, DR. H. S. JACKSON
Editor Section D, PROF. J. R. DYMOND
Editor Section E, DR. J. B. COLLIP
Editor Section F, DR. E. L. HARRINGTON
DR. R. V. V. NICHOLLS

Manuscripts should be addressed:

Editor-in-Chief,
Canadian Journal of Research,
National Research Council, Ottawa, Canada.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 24, SEC. C. OCTOBER, 1946 NUMBER 5

INHERITANCE OF VARIEGATION IN BARLEY[1]

BY T. J. ARNASON[2], J. B. HARRINGTON[3], AND H. A. FRIESEN[4]

Abstract

A strain of variegated barley that originated in a Saskatchewan field produces, on selfing, progeny of which approximately 90% are albino, the remainder striped or variegated with rare full green exceptions. In crosses, variegated ♀ × green ♂ produced 7 albino, 4 striped, and 11 green F_1 plants. The reciprocal cross yielded 1 striped and 41 green F_1 plants. F_2 segregation approximated three green to one of all others (albino and variegated). From some F_3 and F_4 progenies it was inferred that two genetic factor pairs might be segregating. On that hypothesis the dominant hypostatic factor for variegation must be very unstable, mutating at a high rate to white. The peculiarities of inheritance may be explained also, however, on the basis of a combination of gene and (maternal) plastid inheritance. On this interpretation the plastids present in the egg affect the colour of the seedling that develops from it. When green plastids or proplastids are present in the egg, many of them, but not necessarily all, are induced to mutate if the white *w* gene is homozygous, but fewer if the *w* gene is heterozygous. If white plastids only are present in the egg it is probable that the seedling will be an albino regardless of gene content.

Introduction

The inheritance of leaf variegation involving absence of chloroplastids from a part of the mesophyll is not always Mendelian. A large proportion of the reported cases of maternal inheritance involve variegated plants, e.g., *Mirabilis jalapa* (3), *Zea* (1), and *Triticum* (10, 13). Variegation induced by genes and inherited in Mendelian fashion is also known (2, 9). In a third group (5, 11), results are affected by maternal plastid inheritance, gene segregation, and plastid mutation.

Imai (8) suggested that self-reproducing plastids contain plastogenes. Mutation of plastogenes may be induced by certain nuclear genes, and automutation independent of nuclear genes may also occur. When only a portion of the plastids of meristematic, e.g. embryo, cells mutate, segregation of plastids may occur at successive cell divisions resulting in the characteristic green and white sectors. Plastid mutation may occur commonly only at certain stages of the life cycle, e.g. in germ cells or cells of young embryos.

[1] *Manuscript received for publication May 21, 1946.*
Contribution from the Biology and Field Husbandry Laboratories of the University of Saskatchewan, Saskatoon, Sask.

[2] *Associate Professor of Biology.*
[3] *Professor of Field Husbandry.*
[4] *Assistant in Cereal Breeding.*

Imai (5, 6) found in variegated barley that a mutated gene induced plastid mutation. Variegated plants, homozygous for the recessive gene, produced, when selfed, offspring of which about 94% were variegated and 6% were albino. In crosses with green plants the F_1 were green if the mother was green; green or variegated if the mother was variegated. The plastid content of the egg at the time of fertilization affected the phenotype of the hybrid. The F_2 ratio of three green to one variegated indicated that only one gene pair was involved.

In 1940, variegated and albino plants were reported in a farmer's barley field in Saskatchewan. After growing two generations of striped plants, the farmer provided us with seed for use in an inheritance study. The striped plants gave a large proportion of pure white offspring, a small proportion of striped plants, and almost no normal green segregates. The inheritance of variegation obviously was not identical with that reported for barley by Imai (5 to 8). It was decided that the material should be studied cytologically and that reciprocal crosses of striped with green plants were needed to clarify the situation.

Materials and Methods

Seed from variegated barley was space-sown in the cereal breeding nursery at University farm, Saskatoon. The seed from 19 variegated plants grown in the nursery was planted in the greenhouse in a central bed, artificially lighted at night. The light, moisture, and humidity were regulated to resemble field conditions. A second generation was grown both in the greenhouse and the nursery. In each generation the progeny of single plants were separately recorded for leaf colour: that is, whether green, white, or green and white striped. In addition, crosses were made between striped plants and plants of several standard varieties. The F_1, F_2, and F_3 plants were classified for variegation.

The material was studied cytologically. Sections of green, white, and striped leaves were cut after fixation in Craf's fixing solution. Most of these were cut at 15 μ and stained in Heidenhain's haematoxylin or in crystal violet with iodine. Freehand sections of fresh leaves were also studied. Aceto-carmine smears of pollen mother cells were prepared and studied from anthers fixed in alcohol–acetic mixture (3 : 1). Some of the smears were made permanent or semipermanent by mounting in diaphane. Chromosome counts were made and pairing relations observed.

It should be noted here that the leaves of the variegated barley plants have alternating bands of green and white of widely varying widths (Fig. 1). Completely albino leaves and spikes occur occasionally. The leaves are limper and softer to the touch than are ordinary green leaves of barley.

FIG. 1. *Photograph of variegated barley leaves.*

PLATE I

Results

The segregation shown by the progenies of the 19 striped plants grown in the nursery is given in Table I. The proportions of white, striped, and green varied in the different progenies as follows: white, from 75 to 93%; striped, from 7 to 24%, and green, from 0 to 3%. The averages for all the progenies were 84.3% white, 14.5% striped, and 1.2% green.

TABLE I

THE SEGREGATION OBSERVED IN THE PROGENIES OF STRIPED BARLEY PLANTS AFTER SELF-FERTILIZATION IN 1942

Parent plant No.	No. of seeds sown	No. of plants emerged	Number of plants classified as			Percentage of plants classified as		
			White	Striped	Green	White	Striped	Green
1	122	115	98	14	3	85.2	12.2	2.6
2	122	118	108	10	0	91.5	8.5	0.0
3	122	119	103	16	0	86.6	13.4	0.0
4	122	121	100	19	2	82.7	15.7	1.6
5	108	105	89	14	2	84.8	13.4	1.8
6	122	121	91	29	1	75.2	23.9	0.8
7	122	113	101	9	3	89.4	8.0	2.6
8	40	35	32	3	0	92.5	7.5	0.0
9	112	111	100	11	0	90.1	9.9	0.0
10	37	37	33	4	0	89.2	10.8	0.0
11	49	49	45	4	0	91.8	8.2	0.0
12	122	119	99	20	0	89.9	10.1	0.0
13	69	67	52	14	1	77.6	20.9	1.5
14	122	118	98	17	3	83.5	14.4	2.1
15	122	119	92	26	1	77.3	21.8	0.9
16	122	120	91	25	4	75.8	20.2	3.4
17	57	54	50	4	0	92.6	7.4	0.0
18	44	43	39	4	0	90.7	9.3	0.0
19	58	58	48	9	1	82.8	15.5	1.7
Totals		1742	1469	252	21	84.3	14.5	1.2

After two generations of inbreeding at the University, the striped plants produced a somewhat higher proportion of white progeny than was observed in the earlier generations as shown by the results given in Table II. The proportion rose from 84.3 to 93.1% whereas the proportion of striped plants dropped from 14.5 to 6.9% and the proportion of greens fell to zero.

In another test (Part 2 of Table II) the progeny of 17 striped plants selfed for two generations at the University consisted of 359 white and 25 striped plants, that is, 93% of whites and 7% of striped. These figures agree very closely with those of Part 1 of Table II.

In 1945 the progeny (Part 3 of Table II) of another group of individual striped plants separately recorded totalled 897 of which 815 or 90.9% were white, 81 or 9% were striped, and 1 or 0.1% were green. These proportions of white and striped plants are not significantly different from those given in Part 1 of Table II.

TABLE II

THE SEGREGATION OBSERVED IN THE PROGENY OF STRIPED BARLEY AFTER TWO GENERATIONS OF SELF-FERTILIZATION IN 1943 AND 1944

Plant No.	Number of plants	Number of plants classified as		Percentage of plants classified as	
		White	Striped	White	Striped
Part 1					
1	200	189	11	94.5	5.5
5	238	224	14	94.1	5.9
6	250	229	21	91.6	8.4
8	36	35	1	97.2	2.8
10	76	74	2	97.4	2.6
11	55	53	2	96.4	3.6
13	282	263	19	93.3	6.7
15	191	168	23	88.0	12.0
16	234	220	14	94.0	6.0
Totals	1562	1455	107	93.1	6.9
Part 2					
(17 plants)	384	359	25	93.0	7.0
Part 3					
(30 ± plants)	896	815	81	90.9	9.0
Totals	2842	2629	213	92.0	8.0

Seeds from different heads of individual striped plants gave widely fluctuating results. Typical progeny results are given in Table III. Head *d* of *B*245-4 gave 36 white and 10 striped whereas Head *h* gave 43 white and 1 striped. Similarly Head *a* of *B*246-3 gave 20 white and 10 striped as contrasted with Head *c*, which gave 29 white and 1 striped.

The F_1 results of crosses made with plants of commercially used barley varieties are reported in Table IV. The results from reciprocal crosses were not identical, which suggested that direct plastid transmission from mother to offspring influenced the results.

It may be observed from Table IV that of 22 F_1 individuals from striped ♀ × green ♂, only 11 were green, the remainder consisting of four variegated and seven white. This suggests that if mutated white plastids are present in the egg the gene for green is unable to convert them to the green condition. The variegated individuals may have come from eggs having mixed plastids. Since, in the reciprocal crosses, one individual out of 42 was variegated, it is indicated that the gene for variegation even when heterozygous may induce occasional plastid mutation.

TABLE III

HEAD PROGENIES FROM INDIVIDUAL STRIPED PLANTS GROWN IN 1945

Parental plant	Head	White	Striped	Green	Total
*B*245-4	*a*	27	5	0	32
	b	28	0	0	28
	c	25	5	0	30
	d	36	10	0	46
	e	26	4	0	30
	f	26	3	0	29
	g	26	2	0	28
	h	43	1	0	44
	Totals	237	30	0	267
	%	88.8	11.2	0.0	
*B*246-3	*a*	20	10	0	30
	b	29	2	1	32
	c	29	1	0	30
	d	44	2	0	46
	e	28	2	0	30
	f	30	1	0	31
	g	39	8	0	47
	Totals	219	26	1	246
	%	88.9	10.5	0.4	

TABLE IV

THE F_1 RESULTS OF CROSSES BETWEEN STRIPED PLANTS AND GREEN PLANTS OF COMMERCIAL VARIETIES

Cross	Number of plants		
	Green	Striped	White
Striped × Warrior	3	1	1
Striped × Himalayan	2	0	1
Striped × OAC21	0	1	3
Striped × Hannchen	4	0	2
Striped × Regal	2	2	0
Warrior × striped	3	0	0
OAC21 × striped	6	0	0
Hannchen × striped	15	1	0
Regal × striped	17*	0	0

** One of these was listed as pale green.*

The progenies of selfed F_1 plants gave the results presented in Tables V and VI. The results in Table V show the F_2 segregation of crosses of green ♀ × striped ♂. Application of the χ^2 test to the Hannchen × striped results gives a slightly better fit to a 12 : 3 : 1 than to a 3 : 1 ratio. The possibility of a main gene for green vs. white and a hypostatic dominant gene for striped vs. white is suggested. However the fact, proved by F_1 results, that phenotype is not a certain indication of genotype in this material should be kept in mind.

TABLE V

THE F_2 SEGREGATION OF CROSSES OF GREEN PLANTS (FEMALE) × STRIPED PLANTS (MALE)

Cross		Description of F_1 plants	Distribution of F_2 plants			Fit to 3 : 1 ratio of green: others	
Female	Male		Green	Striped	White	χ^2	P
Hannchen	Striped	10 green, 1 striped	147	24	8	4.844	.03
Regal	Striped	One green	4	3	1	2.667	.10
Regal	Striped	One pale green	0	6	0	17.000	.00
Totals (excepting pale green)			151	27	9	3.293	.05

Table VI shows the F_2 segregation of crosses of inbred variegated plants used as females with green plants of standard commercial varieties as male parents. There is a good fit to a 3 : 1 ratio of green to others excepting for the cross variegated × Regal. The χ^2 test for all results excepting those for variegated × Regal gave a P of .70.

TABLE VI

THE F_2 SEGREGATION FROM HYBRIDS BETWEEN INBRED STRIPED FEMALE PLANTS AND MALE PLANTS OF COMMERCIAL GREEN VARIETIES

Cross		F_1 plant	F_2 plants			Green vs others	
Female	Male		Green	Striped	White	χ^2	P
Striped	Warrior	Green	47	6	10	0.053	.80
Striped	Himalayan	Green	166	24	20	1.841	.20
Striped	OAC21	Striped	42	13	7	1.742	.20
Striped	Regal	Green	22	13*	4	7.240	.01
Striped	Regal	Striped	22	10**	4	3.704	.05
Totals (except striped × Regal)			255	43	37	.220	.70

* *Of which three were described as "pale green."*
** *Of which two were described as "pale green."*

The F_2 results of reciprocal crosses are similar in that both give a fair fit to a ratio three green to one of all others. The F_1 and F_2 results considered together suggest that green is partly dominant to the white or striping factor. On this interpretation the inbred variegated plants used in the original crosses are genetically like their sister albino plants homozygous for the recessive "white" gene. That striped F_1 plants possess the factor for green is shown by their green F_2 offspring.

The F_3 segregation from selfed green F_2 plants from the cross Regal (green) × variegated is reported in Table VII. Of 48 F_2 plants classed as green, 10 bred true, 34 segregated to give approximately three green to one of all others, three gave no green offspring, and one gave two green to 13 of others. If the

TABLE VII

THE F_3 SEGREGATION FROM GREEN F_2 PLANTS FROM THE REGAL (GREEN) × STRIPED CROSS

F_2 plant No.		Distribution of F_3 plants			Test for 3 : 1 ratio of green to others		Classification as to 3 : 1 fit*
		Green	Striped	White	χ^2	P	
*B*324	2	13	0	0	4.333	.04	*B*
*B*324	6	9	6	1	2.750	.10	*A*
*B*324	9	13	3	0	0.333	.50	*A*
*B*324	14	9	4	1	0.857	.30	*A*
*B*325	4	10	3	2	0.555	.50	*A*
*B*325	6	0	0	16	50.000	.00	*C*
*B*325	9	13	2	0	1.088	.30	*A*
*B*325	12	14	2	0	1.333	.20	*A*
*B*325	13	10	6	0	1.333	.20	*A*
*B*325	14	16	0	0	5.333	.02	*B*
*B*326	3	11	2	3	0.333	.50	*A*
*B*326	4	0	2	14	50.000	.00	*C*
*B*326	6	11	4	0	0.022	.80	*A*
*B*326	7	10	5	1	1.333	.20	*A*
*B*326	9	10	4	1	1.333	.20	*A*
*B*326	10	15	0	0	4.947	.02	*B*
*B*326	12	0	1	15	50.000	.00	*C*
*B*326	15	12	3	1	0.000	1.00	*A*
*B*327	1	12	3	1	0.000	1.00	*A*
*B*327	2	10	3	2	0.555	.50	*A*
*B*327	6	9	2	4	1.799	.30	*A*
*B*327	8	14	0	1	2.688	.10	*A*
*B*327	9	16	0	0	5.333	.02	*B*
*B*327	12	12	3	0	0.200	.70	*A*
*B*327	14	15	0	0	4.947	.02	*B*
*B*328	2	11	2	0	0.640	.50	*A*
*B*328	3	9	3	3	1.799	.30	*A*
*B*328	4	14	2	0	1.333	.20	*A*
*B*328	5	14	0	0	5.167	.02	*B*
*B*328	6	15	0	0	4.947	.02	*B*
*B*328	7	13	0	2	1.088	.30	*A*
*B*328	8	13	0	0	4.333	.04	*B*
*B*328	9	14	1	1	1.333	.20	*A*
*B*328	12	10	4	1	0.555	.50	*A*
*B*328	15	11	4	2	0.960	.70	*A*
*B*328	16	6	5	0	0.777	.50	*A*
*B*329	2	14	0	0	5.167	.02	*B*
*B*329	5	14	1	0	2.688	.10	*A*
*B*329	8	9	6	0	1.799	.30	*A*
*B*329	10	10	2	0	0.444	.50	*A*
*B*329	12	8	4	3	3.755	.05	*A*
*B*329	15	16	0	0	5.333	.02	*B*
*B*329	16	13	1	1	1.088	.30	*A*
*B*330	3	9	7	0	2.750	.10	*A*
*B*330	5	12	3	1	0.000	1.00	*A*
*B*330	8	2	1	12	30.421	.00	*C*
*B*330	12	11	2	3	0.333	.50	*A*
*B*330	15	13	3	0	0.333	.50	*A*

*"*A*" represents progenies with P values of .05 or higher. "*B*" and "*C*" stand for those with P values below .05. The 10 B progenies appear to be pure breeding greens. The four C progenies are those consisting almost exclusively of albinos.*

four green F_2 plants that produced few or no green offspring are excluded, the ratio obtained is 10 homozygous to 34 heterozygous green F_2 plants. The goodness of fit to a two to one ratio is indicated by χ^2 of 2.23 and P of .15.

The progenies shown in Table VII fall into three general groups on the basis of the χ^2 tests as shown in the last column of the table. The *A* progenies are those that statistically may be considered satisfactory fits to a single factor hypothesis for green. The *B* progenies are the 10 that appear to be true breeding greens. The *C* segregations are four in which the F_2 plants apparently lacked the gene for green colour. Totalling the results for the *A* progenies gives 376 green, 105 variegated, and 35 white. The fit for a dominant green gene is very good, P being .30. In addition there is indication of a dominant hypostatic gene for variegated as the fit to a 12 : 3 : 1 ratio of green, variegated, and white is excellent with a χ^2 of 1.24 and a P of .5. If such a hypostatic gene is assumed there would have to be the further assumption of a very high mutation rate from variegated to white.

In Table VIII the F_3 segregation is given for selfed F_2 variegated plants from the cross Regal × variegated. Five of the 19 progenies had some

TABLE VIII

THE F_3 SEGREGATION FROM STRIPED F_2 PLANTS FROM THE CROSS REGAL (GREEN) × STRIPED

F_2 plant No.	F_3 plants			F_2 plant No.*	F_3 plants		
	Green	Striped	White		Green	Striped	White
*B*323 6	0	1	7	*B*323 10	8	1	5
*B*323 11	0	0	12	*B*323 14	8	1	4
*B*324 13	0	1	11	*B*323 15	12	0	2
*B*325 2	0	0	9	*B*325 11	10	3	2
*B*325 7	0	0	9	*B*326 11	13	3	0
*B*326 2	1	2	13				
*B*326 5	0	0	14	Totals	52	8	13
*B*328 14	0	2	7				
*B*329 11	0	1	15				
*B*329 13	0	1	4				
*B*329 14	0	1	15				
*B*330 4	0	1	15				
*B*330 11	0	4	12				
Totals	—	14	143				

* *These five plants had 52 green and 21 other progeny. The fit to a 3 : 1 ratio is good, χ^2 being 1.10 and P being .5.*

green plants and their segregations both separately and collectively showed good fits to a 3 : 1 ratio for green to others (P being .5). It may be assumed that the five plants are heterozygous for variegation and are genetically like the 34 heterozygous greens listed in Table VII. The results from this group do not support a two factor interpretation. Since striped plants that segregate produce a larger proportion of albino offspring than do segregating green plants, a direct effect of plastid transmission through the egg is indicated.

The cytological study gave the following results. Mesophyll cells of green leaves were found to contain many chloroplastids. Few plastids of any kind were detected in mesophyll cells of white leaves or the white portions of variegated leaves. Many small bodies mostly less than 2 μ in diameter were in these cells. The iodine test for starch on white leaves usually gave no starch reaction. A slight trace of starch was found rarely in leaves of albino seedlings. To determine whether mesophyll cells of "white" tissues were well supplied with plastids capable of depositing starch, the cut tips of detached white leaves were immersed in 10% sucrose solution for 24 to 48 hr., then tested for starch and freehand sections examined. Numerous starch grains of sizes similar to those in green plastids were formed. It is concluded therefore that mesophyll cells of white leaves contain many small colourless plastids. Presumably these plastids are colourless derivatives of mutated chloroplastids.

No visible chromosome irregularity was detected in acetocarmine mounts of pollen mother cells of variegated barley. Seven normal-appearing bivalents were observed in many cells. These were similar to the seven bivalents seen in pollen mother cells of Regal barley. Figs. 2 and 3 represent cells of Regal and variegated, respectively, at diakinesis. A few preparations from F_1 plants of green × variegated have been examined without any chromosome irregularity being noticed. Fig. 4 shows an F_1 microsporocyte with seven bivalent chromosomes.

FIG. 2. *Regal barley. Diakinesis. Seven bivalents.* ×*1000.*
FIG. 3. *Variegated barley. Diakinesis. Seven bivalents.* ×*1000.*
FIG. 4. *F_1 of Regal × variegated barley. Diakinesis. Seven bivalents.* ×*1000.*

Discussion

Characters dependent on plastid characters are of special interest to students of gene physiology. Plastids, like genes or chromosomes, are capable of reproducing their kind by division. Proplastids, which also form plastids, may be directly related by descent to pre-existing plastids or proplastids. Although the plastid can reproduce itself it does not have full control over its own constitution. A mutated nuclear gene may deprive some or all plastids of chlorophyll. According to Rhoades (12) the iojap gene on chromosome seven in corn induces plastid mutation. Not all the plastids mutate so that some leaf sectors are green. The white plastids even in the presence of the normal 'plus' gene do not mutate back to green. In the variegated barley studied by Imai (5, 6) a somewhat similar explanation was put forward.

One of two hypotheses suggested to account for the results obtained in this investigation assumes that the explanation of the peculiarities of inheritance in our variegated barley resembles that given by Rhoades (12) for the inheritance of the iojap character in corn. On this hypothesis (called Hypothesis I) a single gene mutation was responsible for the origin of the variegated and albino phenotypes. Plastid mutation induced by the mutated gene followed by the segregation of plastids in tissues and transmission through eggs is directly responsible for the occurrence of striped leaves and also for the increasing proportion of albinos in successive generations. The other, which will be known as Hypothesis II, assumes two dominant genes for green, one epistatic to the other with rare mutation in the major gene but a high mutation rate in the other gene in the absence of the first one. As both theories include the presence of a main dominant gene the summarized data from Tables V to VIII may be scrutinized in that regard. The pertinent data are as follows:

Table	Cross	Green	Striped	White	*P* values for	
					3 : 1	12 : 3 : 1
V	Hannchen × striped	151	27	9	.05	.06
VI	Striped × 3 varieties	255	43	37	.7	.00
VII	Selfed heterozygotes	376	105	35	.3	.50
VIII	Selfed heterozygotes	52	8	13	.5	.00
Totals		834	183	94	.9	.01
Totals for Tables V and VII		527	132	44	.9	.90

This tabulation does not include any F_2 or F_3 data showing a very poor *P* value for a fit to a 3 to 1 ratio of green plants to others. The combined data show an excellent fit to a 3 to 1 ratio. It is of interest that of the 66 F_2 plants given a progeny test in F_3, four of the 48 recorded as green (Table VII) had the typical "inbred striped" breeding behaviour and five of the 18 recorded as striped (Table VIII) segregated like typical heterozygous greens. The corrected F_2 classification of the Regal × variegated cross is 10 pure for green (*WW*), 39 heterozygous (*Ww*), and 17 typical for striping (*ww*). These results give a *P* of over .5 for a fit to a three to one ratio. The extent of the errors in the classification of F_2 plants is not unusual especially for a character so variable in expression as striping. It seems reasonable that striped plants with a negligible amount of white striping would appear to be green. It is not easy to explain how genetically green plants could appear to be striped unless white plastids were transmitted directly through eggs of a variegated mother or plastid mutation occurred in heterozygous greens.

Now we turn to the results from selfed striped plants and striped × striped crosses. The percentage figures on all the data are brought together in the following tabulation.

Table	Material	Percentages of plants		
		Green	Striped	White
1	19 striped plants	1.2	14.5	84.3
2	9 striped plants	0.0	6.9	93.1
2	17 striped plants	0.0	7.0	93.0
2	30 ± striped plants	0.0	9.0	90.0
3	2 striped plants	0.2	10.9	88.9
8	13 striped plants	0.0	8.9	91.1
	Unweighted averages	0.2	9.1	90.3

Apart from the very large proportion of albino offspring of selfed variegated plants, it is of interest to note that the proportion of whites was higher in the second generation of inbreeding (Table II) than in the first (Table I).

In considering the above figures the F_1 results should be kept in mind. Summarizing from Table IV we have the following F_1 results:

——	Green	Striped	White
Striped × green	11	4	7
Green × striped	41	1	0

The two hypotheses suggested to account for the recorded facts are outlined in some detail below.

Hypothesis I

Inbred variegated and albino plants are genetically alike as far as nuclear genes are concerned. They differ in that variegated plants have two kinds of plastids each reproducing its kind, while albino plants have only mutant white plastids. The egg cells of variegated plants may have all green, mixed, or all white plastids. In the presence of the mutant gene, *w*, mutation is induced in some of the green plastids of eggs or zygotes containing them. Segregation of green and white plastids at successive cell divisions brings about the striped-leaf effect. Since nearly all the F_1 plants were green when the female parent was green but were green, white, or striped when the female parent was striped, it is apparent that the plastids or proplastids present in the egg at the time of fertilization affect the phenotype of the individual that develops from it. Heterozygous individuals may be phenotypically green, white, or striped. Dominance of the gene for green (*W*) is indicated by the close fit to a three green to one of all others in F_2 as well as by the high proportion of green F_1 plants. That the gene *w* may induce plastid mutation at a low rate when in the heterozygous condition is indicated by the rare appearance of striped offspring from the green ♀ × variegated male cross. According to Imai (6) there is no plastid transmission through the males in barley. Striped F_2 plants from green F_1, which produced, when

selfed, green to others in a three to one ratio, also indicate the occasional effectiveness of *w* in heterozygous condition in bringing about plastid mutations.

The relatively high proportion of variegated segregates as compared to the number of albinos in F_2 and F_3 progenies from green heterozygotes may be explained on this hypothesis as follows: the eggs giving rise to these individuals receive green plastids in all cases from the green parent. In order to produce a full albino, all of the plastids must mutate to white early in development. The plastid mutation rate is high enough to accomplish this only in about one-quarter of the individuals. In the remainder only a part of the plastids have mutated.

The wide variation in the proportions of albinos from different heads of the same inbred variegated plant (Table III) may be attributed to plastid segregation. Very little green tissue is present in some heads while others have much. In the latter a considerable proportion of egg cells may be equipped with chloroplastids while in the former few or none have chloroplastids.

As mentioned in the introduction there are a number of reports of maternal plastid inheritance. Of these earlier instances the one most resembling the situation described in this paper is that of Imai (5). Our barley differs most strikingly from Imai's in that it produces on selfing about 90% of albino offspring compared to 10% in his.

Apart from plastids there have been few reports of cytoplasmic inheritance. Cytoplasmic transmission of a male-sterility factor was reported by Rhoades (11) and several other accounts have been reviewed recently by Darlington (4).

Hypothesis II

The presence of a second gene is indicated by the data of Tables V and VII where there is a very good fit to a 3 : 1 ratio for the striped and white plants. The pertinent data show 527 green, 132 striped, and 44 white, which gives an excellent fit to a 12 : 3 : 1 ratio, *P* being .90. Assuming that the factorial constitution of a normal green plant is WWW_1W_1 and that of a striped plant is wwW_1W_1 the difference having occurred originally through a point mutation of *W*, we can arrive at the results of Tables I to IV through mutation in W_1. It would be necessary to postulate that W_1 is relatively stable in the presence of *W* but so unstable in the absence of *W* that mutations of W_1 to w_1 are frequent. In that case a self-fertilized wwW_1W_1 embryo might by mutation become wwW_1w_1, which would still give a striped plant, or even www_1w_1, which would become a white plant. Repeating the selfing of striped plants would involve some wwW_1w_1 plants, which, with a continued high mutation rate, would be expected to produce a very high proportion of whites.

The case in favour of Theory I is supported by the work of Imai and others. On the other hand, Theory II is not unreasonable and should be kept in mind in the case of further work being done.

References

1. ANDERSON, E G Maternal inheritance of chlorophyll in maize. Botan. Gaz. 76 : 411-418. 1923.
2. BEADLE, G. W. Yellow stripe—a factor for chlorophyll deficiency in maize located in the *Pr pr* chromosome. Am. Naturalist, 63 : 189-192. 1929.
3. CORRENS, C. Vererbungsversuche mit blass (gelb) grünen und buntblättrigen Sippen bei *Mirabilis jalapa, Urtica pilulifera* und *Lunaria annua*. Z. Induktive Abstammungs-Vererbungslehre, 1 : 291-329. 1909.
4. DARLINGTON, C. D. Heredity, development and infection. Nature, 154 : 164-169. 1944.
5. IMAI, Y. A consideration of variegation. Genetics, 13 : 544-562. 1928.
6. IMAI, Y. Variation in the rate of recurring plastid mutations in *Hordeum vulgare* caused by differences in the sowing times. Genetics, 20 : 36-41. 1935.
7. IMAI, Y. Recurrent auto—and exomutation of plastids resulting in tricolored variegation of *Hordeum vulgare*. Genetics, 21 : 752-757. 1936.
8. IMAI, Y. The behaviour of the plastid as a hereditary unit. Cytologia, Fujii Jubilee Volume: 934-947. 1937.
9. JODON, N. E. Inheritance and linkage relationships of a chlorophyll mutation in rice. J. Am. Soc. Agron. 32 : 342-346. 1940.
10. PAO, W. K. and LI, H. W. Maternal inheritance of variegation in common wheat. J. Am. Soc. Agron. 38 : 90-94. 1946.
11. RHOADES, M. M. The cytoplasmic inheritance of male sterility in *Zea mays*. J. Genetics, 27 : 71-93. 1933.
12. RHOADES, M. M. Genic induction of an inherited cytoplasmic difference. Proc. Natl. Acad. Sci. 29 : 327-329. 1943.
13. UMAR, S. M. A case of maternally inherited variegation in wheat. Indian J. Genetics Plant Breeding, 3 : 61-63. 1943. *Abstracted in* Biol. Abstracts, 19 : 449 (No. 4241). 1945.

INVESTIGATIONS OF DECAY IN WESTERN RED CEDAR IN BRITISH COLUMBIA[1]

By D. C. Buckland[2]

Abstract

Investigations of decay in *Thuja plicata* D. Don. in British Columbia have shown that the major heart-rotting fungi of living western red cedar on the Coast, in decreasing order of importance, are *Poria asiatica* (Pilát) Overh. (brown cubical pocket and butt rot), *Poria albipellucida* Baxter (white ring rot), *Fomes Pini* (Thore) Lloyd (white pitted trunk rot), *Merulius* sp. (brown crumbly butt rot), and *Poria subacida* (Peck) Sacc. (spongy white rot). In the Interior of the Province they are *Poria asiatica*, *P. Weirii* Murr. (yellow ring rot), *Fomes Pini*, *Polyporus balsameus* Peck (brown cubical butt rot), *Merulius* sp., and *Poria subacida*. Other fungi attacking the heartwood of living trees less extensively are *Fomes annosus* (Fr.) Cke., *F. nigrolimitatus* (Romell) Egel., *F. pinicola* (Sw.) Cke., *Armillaria mellea* (Fr.) Quél., *Omphalia campanella* (Fr.) Quél., *Polyporus Schweinitzii* Fr., and *Coniophora cerebella* Pers.

On the areas investigated, loss through decay in stands 50 to 450 years of age never exceeded growth increment. It was indicated that decay in younger stands was of importance. Loss through decay and incidence of infection was appreciably higher in the Interior than on the Coast. No reliable external indications of decay in the standing tree were found.

Deterioration in felled trees was caused by *Poria Weirii*, *P. albipellucida*, *P. subacida*, *Armillaria mellea*, *Omphalia campanella*, *Fomes annosus*, *F. nigrolimitatus*, and *F. pinicola*. *Polyporus cuneatus* (Murr.) Overh., and *Hymenochaete tabacina* (Sow.) Lév. were the major decay organisms of cedar sapwood and slash. *Polyporus abietinus* (Dicks.) Fr., *P. versicolor* (L.) Fr., *P. semipileatus* Peck, *P. hirsutus* (Wulf.) Fr., and *Poria isabellina* (Fr.) Overh. were restricted in distribution or of irregular occurrence.

To assist in the identification of cultures all the major heart-rotting organisms were grown on tannic and gallic acid media. Seventy-seven species of Basidiomycetes were collected on living and dead *Thuja plicata* in British Columbia from 1943 to 1945.

Introduction

The decays of western red cedar, *Thuja plicata* D. Don., have received little attention in the past. This apparent neglect probably arose because of the well-established reputation of cedar heartwood for high resistance to decay under service conditions, with the resultant lack of interest in the fungi that occurred in living trees. However, the ever-increasing shipments of cedar from British Columbia to distant markets, as lumber, poles, or other products, has aroused interest in the cause of certain discolorations in the heartwood. In response to requests that a study of these discolorations be carried out, an investigation was initiated in 1943.

Western red cedar is one of the most important softwoods of British Columbia. The British Columbia Forest Branch has reported (19) that

[1] *Manuscript received May 2, 1946.*

Contribution from the Graduate School, Yale University, New Haven, Conn., and from the Division of Botany and Plant Pathology (No. 868), Science Service, Department of Agriculture, Ottawa. This paper constitutes part of a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the Graduate School, Yale University, May, 1945.

[2] *Assistant Forest Pathologist, Dominion Laboratory of Forest Pathology, 714 Belmont Building, Victoria, B.C.*

there is a total of 24,597 million bd. ft. of accessible cedar of merchantable size in the province. In total volume this species ranks second only to western hemlock. The average annual cut (9) for the past 10 years amounted to 567 million bd. ft., standing second to that of Douglas fir.

Prior to this investigation little was known of the occurrence and relative importance of the various fungi causing decay of western red cedar. During recent years a number of attempts have been made to determine by cultural methods the identity of the heart-rotting fungi, particularly those causing the brown cubical decays, but these have been abandoned or set aside because of the difficulty in isolating the organisms concerned. On reading the literature on the decays of cedar it is found that only one of the major decays is ascribed to a known fungus, that being the yellow ring rot caused by *Poria Weirii* Murr. Other observational reports were published on the occurrence of *Fomes Pini* (Thore) Lloyd, *Polyporus Schweinitzii* Fr., *Armillaria mellea* (Fr.) Quél., and *Poria subacida* (Peck) Sacc. on cedar, but no data were collected on the relative importance of these wood-rotting organisms. The first phase of this investigation was, therefore, to determine the fungi responsible for the decays and to ascertain the relative importance of each.

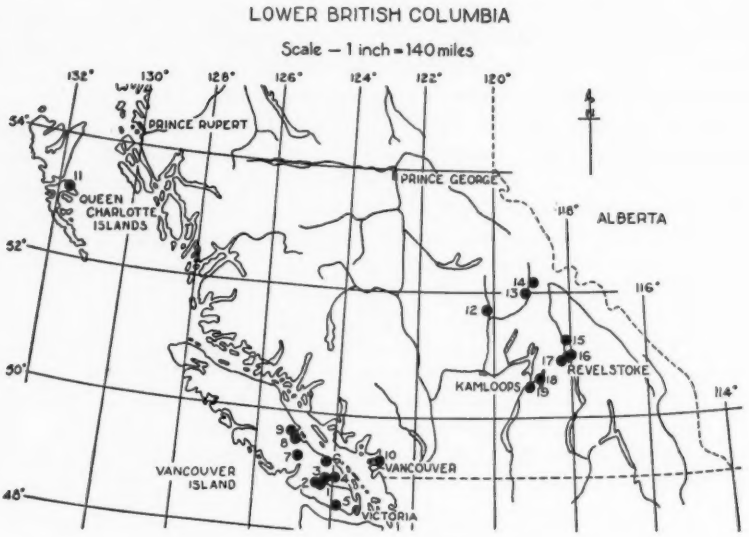
A total of 615 trees was examined in 11 localities on the Coast*, and 110 trees in eight localities in the Interior (Text-fig. 1).

Methods of Study

As the first phase of this study was to be an attempt to separate and name the fungi causing decay in western red cedar, and to become familiar with the gross characteristics of the decays, the choice of areas was not the best for collecting data to determine the relative importance of the wood-destroying fungi in relation to age of the host. To obtain as complete a picture of the distribution of the wood-destroying fungi as possible, the localities for study were chosen over a wide range. The governing features in the choice of an area were the numerical abundance of cedar, the freshness of cut, and the accessibility of the stand to laboratory facilities. These requirements were most satisfactorily met in localities where pole-cutting operations were being carried out.

A 100% cruise of all felled western red cedar on the areas under investigation was attempted. Local topography was occasionally so steep or rugged that as low as 80% of the trees were inspected. On every tree measurements were taken of the stump height, stump diameter, and the diameter at critical intervals along the stem. A count of the annual rings was made at stump height and the total age calculated by estimating the number of years it took the tree to grow to stump height. The frequent occurrence of hollow butts made this age count impossible on many trees. In such cases the annual rings were counted as close to the butt as possible and the estimated time the

* *The term "Coast" is used to refer to all areas of British Columbia west of the Coast Range, while "Interior" refers to all areas east of these mountains.*



STUDY OF DECAY IN WESTERN RED CEDAR

LOCALITIES INVESTIGATED

1 - Cowichan Lake
2 - Nixon Creek
3 - Meade Creek
4 - Copper Canyon, Chemainus
5 - Muir Creek, Sooke
6 - Bonell Creek, Nanoose Bay
7 - Elsie Lake, Alberni
8 - Tsable River, Fanny Bay
9 - Courtenay District
10 - Port Moody Cedar Sawmill
11 - Skidegate Lake
12 - Clearwater River
13 - Blue River
14 - Upper Thompson River
15 - LaForme Creek
16 - Illecillewaet River
17 - Begbie Creek
18 - Mabel Lake
19 - Shuswap River

TEXT-FIG. 1. *Localities investigated in the study of decay in western red cedar.*

tree required to grow to this height was added, giving a reliable approximation of the total age.

Complete information was noted in the field on the type of decay, the extent, similarity to other infections in the area, probable entrance point, and the extent, character, and age of any wounds or deformities. Whenever possible a large collection of the decay in incipient and advanced stages was made. The specimens of decay were removed to the laboratory as shortly after collection as possible so that culturing of the organisms could be carried out while the specimens were still fresh.

All trees were plotted on British Columbia Forest Service Form 6503, a form similar to the United States Forest Service Form 558*a*, and the cubic volumes were measured from a standard stump height of 3 ft. to a 6-in.

merchantable top for all pole timbers, and a 12-in. merchantable top for saw-log timbers. This computation involved the measuring of the area between the outline of the plotted tree and the diameter and height axes with a planimeter, and multiplying the result by a conversion factor. All measurements were made twice, and if the two readings did not check to within 1%, the measurements were repeated. Computations were carried to two decimal places, and the resulting figures were taken to the nearest tenth of a cubic foot. This procedure was repeated for the measurement of the decay column in infected trees.

In all cases where doubt existed concerning the identity of the decay in the field, a specimen was taken to the laboratory for inspection and culturing. In the laboratory all decay specimens giving similar organisms in culture were grouped. Those from which no cultures were obtained, but which closely resembled a typical specimen of known rot, were grouped with the known decay for computing the relative importance of this decay.

Description of the Decays

For the purpose of this study, the decays were separated into white and brown rots, depending on the reaction of the causal fungus on tannic and gallic acid media (11). In general the organisms causing white rot give a positive reaction on tannic and gallic acid media, staining the media brown, while those causing brown rot give no reaction on these media. Thirteen species of fungi have been found to cause decay of the heartwood of living cedar. Of these, eight cause white rots and five brown rots.

White Rots

Four fungi capable of producing white rots of importance and four of possible significance in localized areas have been found in western red cedar. The major wood-destroying organisms have been identified as *Poria albipellucida* Baxter, *Poria Weirii*, *Fomes Pini*, and *Poria subacida*. The four of secondary importance were identified as *Fomes annosus* (Fr.) Cke., *Armillaria mellea*, *Omphalia campanella* (Fr.) Quél., and *Fomes nigrolimitatus* (Romell) Egel.

White Ring Rot (Poria albipellucida) and Yellow Ring Rot (Poria Weirii)

The decays caused by *Poria albipellucida* and *Poria Weirii* have been found to be very similar in gross appearance. This similarity is apparently the reason that the decay caused by *P. albipellucida* has been almost unrecognized in the past, all the ring rot of cedar being attributed to *P. Weirii*. That an organism other than *P. Weirii* was known to cause a ring rot in cedar on the Coast was indicated by Eades and Alexander (12) and others.

P. albipellucida (3) (Pl. I, Figs. 1 and 2) and *P. Weirii* (21) (Pl. II, Fig. 1) both cause a white to yellow ring rot of living western red cedar. The incipient stage is first manifested by a yellowing in the reddish-brown heartwood, the wood remaining firm. With *P. albipellucida* decay, occasionally a

bluish, marginal discoloration or the appearance of darker, reddish stains at the limits of infection are observed. Later the wood becomes much softened and the discoloration is more evident. In the advanced stage the annual rings separate, the earlywood decaying more readily than the latewood, and the typical, laminated ring rot appears. In this stage the rots caused by the two fungi can be separated. *P. albipellucida* ring rot very commonly shows conspicuous, white, mycelial flecks between the laminae, and the wood surface shows minute, elongate, fine pits (Pl. I, Fig. 3). The late advanced stage is a typical ring rot, rarely fibrous in nature (Pl. I, Figs. 6 and 7). On the other hand, *P. Weirii* ring rot sometimes shows small patches or a thin web of setal hyphae, dark brown in colour, between the laminae, and the wood shows long striations, or thin, closely-packed pockets, which, before the complete separation of the laminae, contain white material, probably cellulose products (Pl. II, Figs. 2 and 3). This striation of the laminae gives the advanced rot a fibrous texture. The pole-cutting operators in the Interior refer to *P. Weirii* rot as 'paper rot', as the advanced stage is a mass of thin, flexible laminae (Pl. II, Fig. 4). On the Coast this term has not been applied to *P. albipellucida* decay, as the final stage is coarsely laminated or a crumbly, amorphous mass. Insects, particularly ants and termites, seem to favour this latter rot and are commonly found associated with it.

Present evidence based on identification of decay organisms by cultural methods or by direct examination of the rots (Table I) suggests that, as an important heart-rotting organism of cedar, *P. albipellucida* is limited to the Coast, while the major damage by *P. Weirii* is in the Interior. Although both

---

PLATE I. FIGS. 1 to 7. *Poria albipellucida on western red cedar.*

FIG. 1. *Sporophore on slash.* × 1.

FIG. 2. *Sporophore on a railroad tie.* × 1.

FIG. 3. *Tangential view of heartwood showing the advanced stage of white laminate rot with mycelial flecks.* × ½.

FIG. 4. *Cross section through the butt of a freshly cut pole showing the incipient stage of white laminate rot.*

FIG. 5. *The same pole after seasoning showing the formation of ring shakes in the heartwood.*

FIG. 6. *Longitudinal section of cedar showing the advanced stage of white laminate rot.* × ½.

FIG. 7. *Cross section through a log showing the incipient and advanced stages of white laminate decay.*

PLATE II. FIGS. 1 to 4. *Poria Weirii on western red cedar.*

FIG. 1. *Sporophore showing stratification, on the lower side of a log.* × ⅓.

FIG. 2. *Tangential view of heartwood showing the advanced stage of yellow ring rot.* × ½.

FIG. 3. *Longitudinal section of a young cedar showing the heartwood completely rotted, and a mat of setal hyphae over the surface of the exposed infected wood.* × ½.

FIG. 4. *Longitudinal section of a log showing the advanced stage of yellow ring rot.*

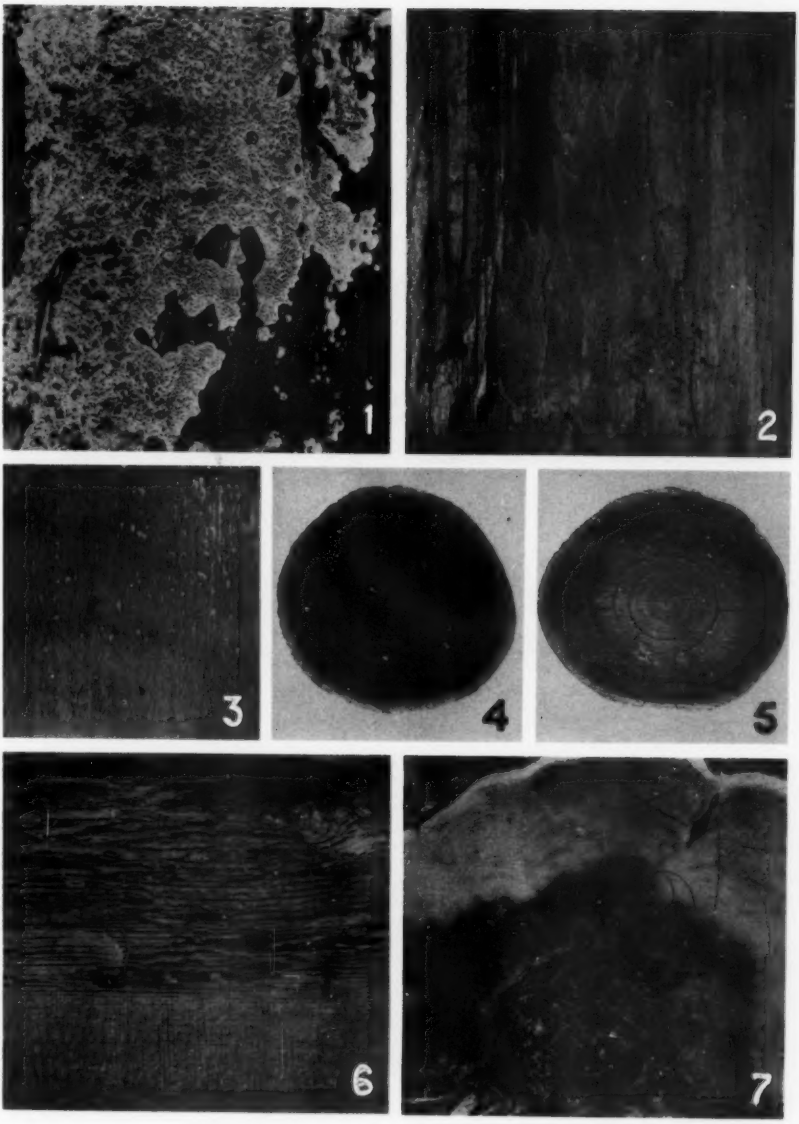
FIGS. 5 to 7. *Poria subacida on western red cedar.*

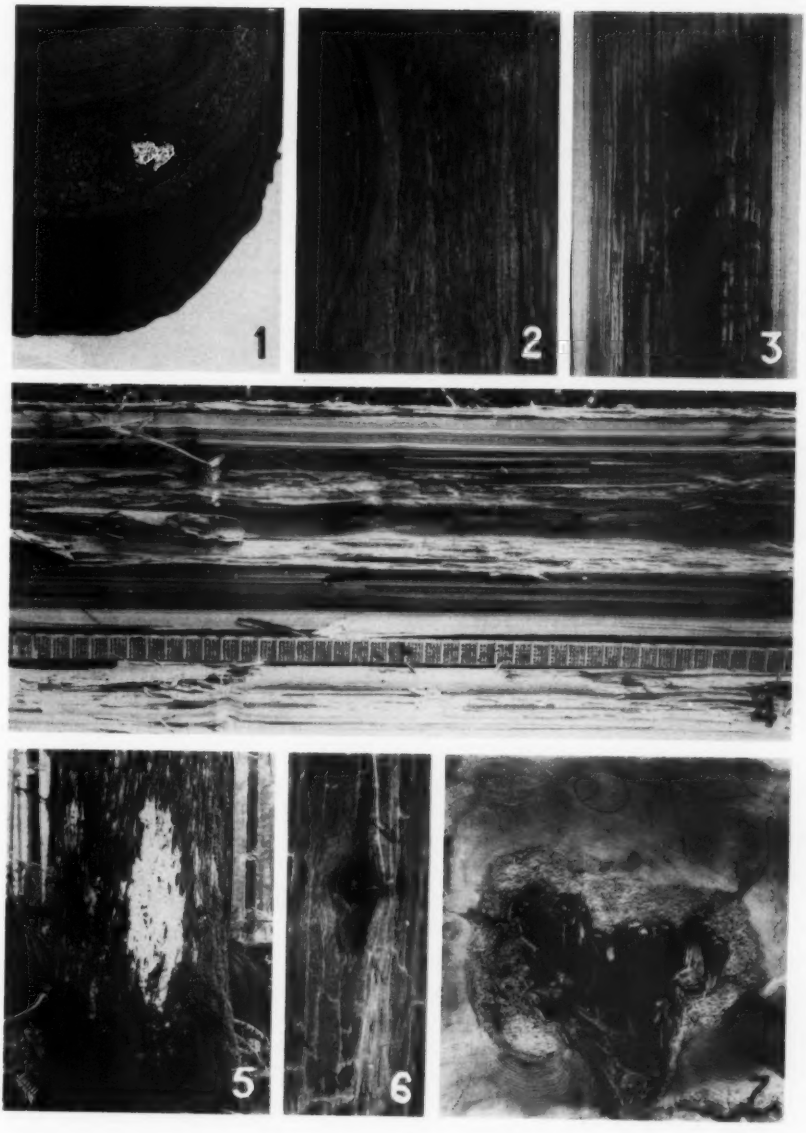
FIG. 5. *An annual sporophore growing on the side of a living tree.*

FIG. 6. *Tangential view of heartwood showing the advanced stage of white spongy rot with white rhizomorph-like strands between the laminae.* × ⅓.

FIG. 7. *Cross section through a log showing the incipient and advanced stages of white spongy rot.*

PLATE 1




1
2
3
5
6

these organisms are present in both regions, it is doubtful whether *P. albipellucida* attacks living trees in the Interior, while *P. Weirii* attacks such trees only to a limited extent on the Coast, occasionally causing damage in young growth (18) and mature trees. The reason for the regional activity of these organisms is obscure.

TABLE I

REGIONAL AND HOST AGE-CLASS DISTRIBUTION OF ISOLATIONS AND AUTHENTIC RECORDS OF *Poria Weirii* AND *Poria albipellucida*

Age class of infected trees	Number of isolations and authentic records			
	Poria Weirii		*Poria albipellucida*	
Years	Coast	Interior	Coast	Interior
50	1	2	2	—
100	—	—	2	—
150	—	3	11	—
200	—	10	32	1
250	—	17	16	—
300	1	3	—	—
350	—	1	5	—
400	—	—	3	—
Total	2	36	71	1

White Pocket Rot (Fomes Pini)

White pocket rot or conk rot, caused by *Fomes Pini* (25), was found to be of some importance in all cedar stands (Pl. III, Figs. 1 and 2). The description of the decay as given by Baxter (5), Boyce (8), and Hubert (15), for other hosts applies for the most part to cedar.

The incipient stage is usually not easily distinguished beyond the advanced rot column, occasionally appearing as a slight paling of the heartwood. On the lateral margins of the advanced decay, however, the incipient rot is frequently associated with a narrow, bluish to reddish-brown line or band (Pl. III, Fig. 3). Occasionally in basal infections the decay did not appear typical of *F. Pini*, but was a clearly-marked band of yellow discoloration containing only fine pits and was commonly accompanied by brown zone lines.

Spongy White Rot (Poria subacida)

A spongy white rot or stringy butt rot of cedar is caused by *Poria subacida* (Pl. II, Fig. 5). This decay is referred to extensively in literature as a spongy rot of *Thuja occidentalis* Linn. and a feather rot of *Abies balsameus* Mill. in the forests of Northeastern America (2, 17, 23, 24).

The incipient stage is usually limited to a thin margin about the advanced decay, where the wood becomes more deeply coloured. The advanced decay, while somewhat laminated in appearance, is readily distinguished from the ring rots of red cedar by the formation of irregularly-shaped pockets, which may coalesce to form a continuous mass of white fibres in the earlywood.

Black flecks sometimes appear in these white fibres. The laminae, on separation, frequently show patches or sheets of white to cream mycelium or rhizomorph-like strands (Pl. II, Fig. 6). In the final stages of decomposition the wood is a feathery to spongy mass, commonly containing numerous secondary decay fungi (Pl. II, Fig. 7). The great majority of infections take place through basal wounds or injured roots.

Minor White Rots

The spongy butt rot of living western red cedar caused by *Fomes annosus* (4) has been found during the course of this study on young trees 35 to 50 years of age. The fungus is apparently of little importance as a wood-rotting organism of mature cedar, but may prove of some interest in young stands. Day* reports that this organism has been observed to cause a butt rot of cedar in a number of places in England.

Armillaria mellea (25), the honey mushroom, causes a root and butt rot of cedar of some importance in localized areas. In the University of British Columbia forest, Vancouver, a number of young cedars were reported by Dr. J. E. Bier to have been attacked and girdled by this fungus. In the Blue River area (Text-fig. 1, Locality 13) a number of trees of about 200 years of age were found with the butts completely rotted into a yellow-brown, spongy mass, or with one to several of the roots and a small portion of the butts in a similar condition. *A. mellea* was fruiting abundantly on the decayed wood of these trees, occasionally growing from the infected tree one to four feet above the ground. Although *A. mellea* is capable of causing extensive damage in a restricted area it is, in general, unimportant on cedar.

Omphalia campanella (13, 20), the little bell *Omphalia*, reported occurring on decayed coniferous logs and stumps, may prove of some interest as a fungus possibly causing decay of living trees. This minute mushroom, the cap 0.25 to 0.75 in. broad, and stipe 0.5 to 1.5 in. long, produces 'aerial rhizomorphs' in culture and in decay pockets (Pl. III, Figs. 4 and 5). The fungus has been

* *Correspondence dated May 2, 1944.*

FIGS. 1 to 3. *Fomes Pini on western red cedar.*

FIG. 1. *Longitudinal section showing the advanced stage of white pitted trunk rot and a sporophore of the causal fungus growing from a branch stub.* × ½.

FIG. 2. *Sporophores of the white pitted trunk rot fungus on the end of an infected log.* × ⅓.

FIG. 3. *Longitudinal section of heartwood showing the incipient stage with discoloration, and the advanced stage, of white pitted trunk rot.*

FIGS. 4 and 5. *Omphalia campanella on western red cedar.*

FIG. 4. *Sporophores growing from decayed heartwood.* × ⅙.

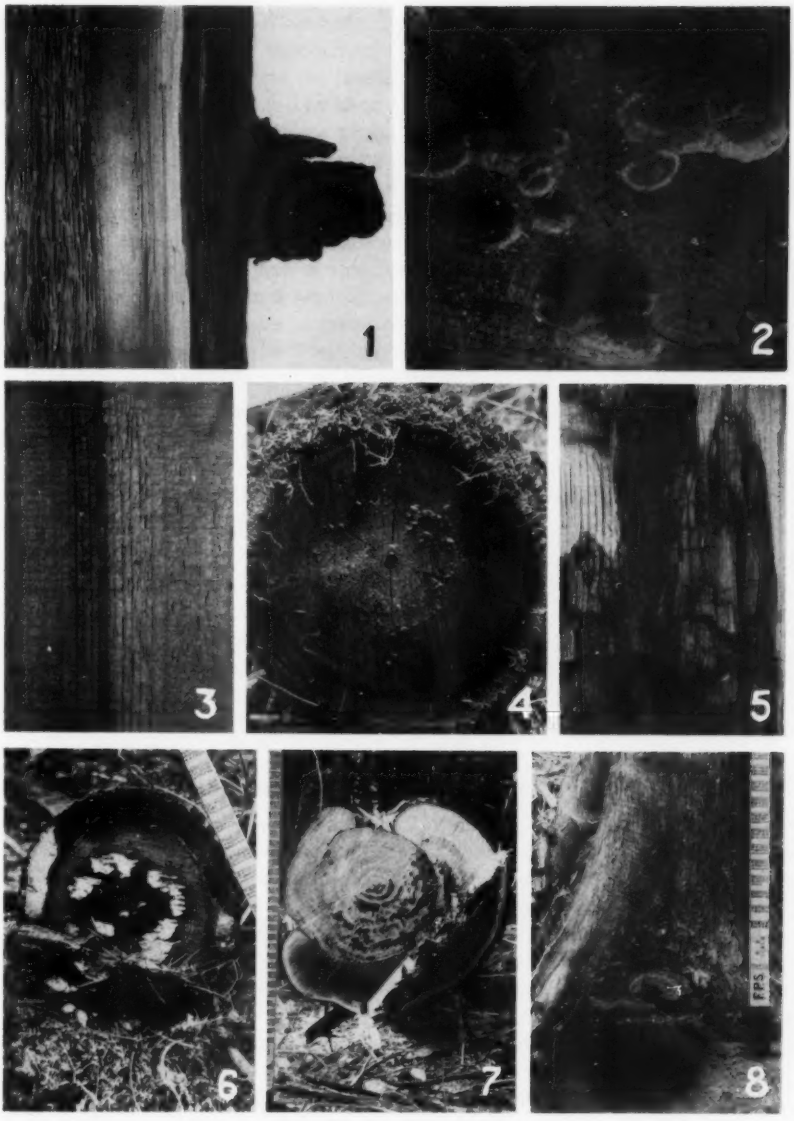
FIG. 5. *Longitudinal section of a living tree showing the black 'aerial rhizomorphs' of the fungus in a pocket of brown cubical rot caused by Poria asiatica.* × ⅓.

FIGS. 6 and 7. *Polyporus balsameus on western red cedar.*

FIG. 6. *Sporophores growing from brown cubical butt rot.* × ⅛.

FIG. 7. *Cross section through the butt of a tree showing piped or tubular rot caused by the fungus.*

FIG. 8. *Polyporus Schweinitzii sporophores on the root of a living western red cedar.*


1
2
3
4
5
6
7
8

found associated with the decays of the white rot fungi, *Poria albipellucida*, *P. Weirii*, and *Fomes Pini*, and the brown cubical rot fungus, *Poria asiatica*. Whenever the fungus has been isolated from the incipient decay associated with a brown cubical rot, invariably 'aerial rhizomorphs' have been found scattered or densely packed throughout the brown cubes. Where this fungus was found with the white rots, no 'aerial rhizomorphs' have been found, but the decay was more yellow-brown than the normal white rot and contained fine, brown zone lines. It seems probable that this fungus is a secondary wood-destroying organism only, growing in association with one of the primary rot fungi. It has been cultured on several occasions from as high as 15 feet up the heartwood of a living tree, however, in pockets with brown cubical rot, and from the upper limits of the incipient decay of *P. Weirii* and other white rots. After authentic cultures of this fungus had been obtained from rot in cedar heartwood and also from associated fruit bodies, similar cultures isolated from black spruce and balsam fir pulpwood bolts in the East, and Douglas fir and living Sitka spruce in the West, were identified as this species. Further investigations on the activity of this fungus are required.

Fomes nigrolimitatus, causing a white pocket rot, has been found by R. E. Foster on the Queen Charlotte Islands and on Vancouver Island in living cedar. This fungus has not previously been reported as occurring in living trees. Hubert (15, p. 381) mentions dead material of western red cedar as one of the most common hosts of this fungus.

Brown Rots

During the course of this study six fungi have been found associated with brown cubical rots of living western red cedar. Two of these, *Poria asiatica* (Pilát) Overh. and *Polyporus balsameus* Peck, are of considerable importance. One, tentatively identified as a species of *Merulius*, is probably of some consequence and three others, *Fomes pinicola* (Sw.) Cke., *Polyporus Schweinitzii*, and *Coniophora cerebella* Pers., occur infrequently.

Brown Cubical Pocket and Butt Rot (Poria asiatica)

The decay caused by *Poria asiatica* (22, 23), known as "black rot" throughout the Interior, is responsible for the greatest loss in living western red cedar (Pl. IV, Fig. 1). *P. asiatica* causes a brown cubical butt rot and brown cubical pocket trunk rot of living cedar. The incipient stage is easily distinguished from uninfected wood, and extends several feet above the advanced decay column. In this early stage the wood becomes straw-coloured to pale yellow-brown, softened, 'dead' in appearance, and presents a marked contrast to the normal, reddish-brown heartwood (Pl. IV, Fig. 2). In the advanced stage the infected wood turns light brown to brown, becomes brittle, and breaks along shrinkage cracks to form small cubes, the surfaces of which are frequently covered with felt-like mats of white mycelium (Pl. IV, Fig. 5).

In the late stage of decay the type and size of the rot column varies considerably. Frequently a piped or tubular rot was found on the stump, or a

solid mass of decay that did not always appear to be a more advanced stage of the piped condition. Higher in the trunk these two types of basal decay either developed into typical pocket rot or remained an unbroken column of decay. It was found that where the continuous column of decayed wood was present, the infection was usually limited to the basal 4 to 15 feet, while in trees where the pocket rot was present the infection spread from 6 to 80 or more feet up the bole of the tree, growing out the branches in severe infections (Pl. IV, Figs. 4, 7, 8, and 9). In the latter type of decay, pocket rot, the pockets were irregular in shape, 0.5 to 6 in. wide, and 1 in. to 2 ft. in length, separated at times by one to several feet of discoloured wood, or growing together to form a large irregular pocket of decay (Pl. IV, Fig. 5). Variations in the size and type of pocket were very noticeable, and were probably caused by certain inherent properties of the cedar wood hindering, or otherwise affecting, the growth of the attacking fungus. One infrequent type of decay showed pockets varying from minute to 0.5 by 3 in. in size, appearing in a discoloured, infected core that was decidedly harder than normal cedar wood (Pl. IV, Fig. 3).

P. asiatica was commonly found in association with one of the major white-rot organisms. In such cases both rots involved were usually separable. Where *P. asiatica* was growing with *P. albipellucida* or *P. Weirii*, the former fungus usually grew through the wood already decayed by the white-rot fungus, and on into the sound wood. Where *P. asiatica* and *Fomes Pini* were growing in the same tree, however, the two fungi grew together commonly with no clear line of demarcation between them, resembling in many respects the condition reported by Boyce in his study of dry rot in incense cedar (6).

Brown Cubical Butt Rot (Polyporus balsameus)

As *Polyporus balsameus* was responsible for but few of the brown cubical rot infections studied, being found only on trees in the North Thompson and Revelstoke regions in the Interior, only a tentative description of the decay can be presented at this time (Pl. III, Fig. 6). In the incipient stage the decay caused by this organism is a straw to yellow-brown discoloration of the heartwood. The advanced stage is light brown to brown in colour, breaking into fairly large cubes, or into a split crumbly mass, commonly covered with a

---

Poria asiatica on western red cedar.

FIG. 1. *Sporophores on the end of a log.* × ⅛.

FIG. 2. *Longitudinal section of a tree showing the light heartwood with incipient decay and brown cubical rot caused by the fungus.* × ⅛.

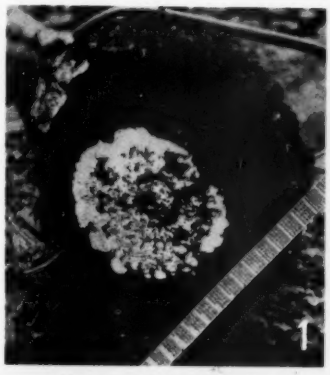
FIG. 3. *Longitudinal section showing small pockets of decay caused by the fungus.* × ½.

FIG. 4. *Cross section 30 feet above the butt of an old tree showing pocket or tubular rot caused by the fungus.*

FIG. 5. *Longitudinal section of a tree showing pocket rot caused by the fungus.*

FIG. 6. *Longitudinal section of a small oppressed tree showing the heartwood completely decayed by the fungus.* × ½.

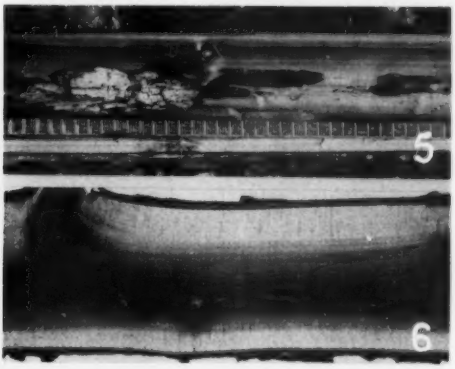
FIGS. 7 to 9. *Brown cubical pocket rot in a pole tree. Fig. 7, 3 feet from ground-level; Fig. 8, 20 feet from ground-level; Fig. 9, 35 feet from ground-level.*

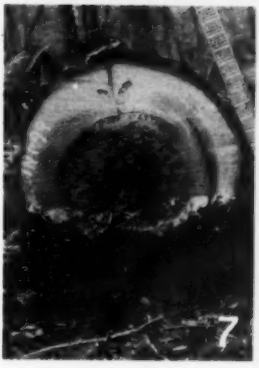

1

2

3

4


5
6


7

20°
8

35°
9

thin weft of white mycelium. In three of the infected trees, the infection courts were either small basal wounds or injured roots, and in each of these cases the rot in the end of the butt log appeared typical of this fungus (14, 17), a large, brown cubical rot. In the other three infected trees, where the infection courts were large basal wounds, the rot appeared as a piped or tubular decay in the basal end (Pl. III, Fig. 7). Higher in the trunk the decays arising from both types of infections were very similar in all respects.

Although this fungus was rarely obtained from decay, it seems possible that it is more frequent than these cultures would indicate. If the organism is a butt rot in its usual manifestation, then it is quite possible that a number of the butt rot infections inspected were caused by this fungus, but the large number of secondary organisms present in these infections makes it impossible in many instances to determine this by cultural methods. A further study of fresh specimens may indicate the relative importance of *P. balsameus* as a brown cubical rot of cedar.

Brown Crumbly Butt Rot (Merulius)

A fungus found by Dr. M. K. Nobles* to be very similar in growth habits in culture to a species of *Merulius* has occurred in sufficient frequency to warrant attention as a butt-rotting organism of cedar. The incipient stage was usually not clearly indicated, but on occasion a yellow-brown discoloration was found. In the advanced stage considerable variation was evident, but the typical form appeared as a crumbly, light-brown to brown decay, breaking on shrinkage cracks to form large cubes or segments. While most infections resulted in typical butt rots, this fungus was also found entering scars and producing narrow, short, crescent-shaped rot columns in which the brown cubes were usually small and very brittle.

Minor Brown Cubical Rots

Fomes pinicola, the red belt fungus, has been isolated from a light-brown cubical trunk rot of living cedar during the course of this study. One collection and one observation of the sporophores on living trees have been made at Vancouver, B.C. On the Coast *F. pinicola* undoubtedly does occur as a decay of living western red cedar but is of minor importance.

Polyporus Schweinitzii, the velvet-top fungus, has long been considered as a possible cause of the brown cubical butt rot of cedar, most of which has been found to be the result of attack by *Poria asiatica*. One culture of this organism from cedar at Vancouver is mentioned by Childs (10), but this culture cannot be traced at present. The fruiting bodies of *P. Schweinitzii* were found on cedar only once in British Columbia, these being attached to the root of a tree 120 years of age (Pl. III, Fig. 8). Although this fungus attacks western red cedar, as reported by Weir (25), it will likely be found to be only of minor importance.

Coniophora cerebella, cultured from decay during the course of this investigation, apparently is capable of causing decay in living western red cedar.

* *Correspondence dated 1943.*

Cartwright* reports that the only serious cases of decay in cedar timbers in England have been caused by this fungus. The presence of *C. cerebella* in living trees is, therefore, of considerable interest.

Entrance of the Decays

Basal wounds and fire scars are the most important avenues for the entrance of decays into living western red cedar. In all localities investigated these injuries were the entrance point of over 90% of the infections. Other points of infection worthy of note, however, are 'stag-head' tops, branch stubs, 'dry-side', and injured roots.

In certain of the dryer areas on the Coast, fire scars were of considerable importance in exposing heartwood to infection, while basal wounds caused by other mechanical decorticating agencies were very frequent generally. In nearly all the localities inspected, but particularly the moister regions of the Interior, basal wounds of doubtful origin were found. These wounds appeared externally as depressed areas in the bark (Pl. V, Fig. 2), limited to a small fraction of the circumference to cases of almost complete girdling. On examination it was found that the cambium had been killed beneath the bark. No correlation was found with any natural causative factor.

Although nearly all scars inspected showed indications of decay, the ability of cedar to heal wounds is very great. A number of cases were observed where the cambium had been killed for over 50% of the circumference, and complete healing had taken place in a relatively short time. In many of these cases excluded hearts were found. Here the callous tissue, rather than growing over the scarred heart, had grown to exclude the heart as illustrated in Pl. V, Figs. 3 and 4, leaving an ever-present entrance for infection. The well-known infolded or fluted base of cedars appears to be chiefly the result of numerous healed basal wounds (Pl. V, Fig. 1). It was observed that all trees inspected, the stumps of which showed no scars were present at any time throughout the life of the trees, were somewhat 'swell-butted', but showed none of the deep infolding or fluting.

* *Correspondence dated May 4, 1944.*

FIGS. 1 to 4. *Entrance of decay in western red cedar.*

FIG. 1. *Cross section of part of the butt of a tree showing the bark seam formed in healing a wound infected with Poria albipellucida.*

FIG. 2. *Cross section of three basal wounds caused by an unknown physical or mechanical agency. Three small sections of the cambium are living.*

FIG. 3. *Illustration of a cross section of a tree at stump height showing how the heart may be excluded laterally during the healing of an extensive wound.*

FIG. 4. *Illustration of a cross section of a tree at stump height showing how the heart may be excluded centrally during the healing of two or more extensive wounds.*

FIG. 5. *Poria albipellucida on malt agar at the end of six weeks.*

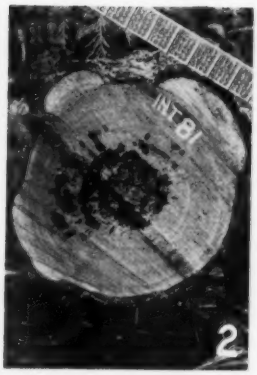
FIGS. 6 to 8. *Merulius sp. in culture.*

FIG. 6. *Merulius "A" on potato-dextrose agar at the end of six weeks.*

FIG. 7. *Merulius "A" on gallic acid medium at the end of one week.*

FIG. 8. *Merulius "B" on gallic acid medium at the end of one week.*

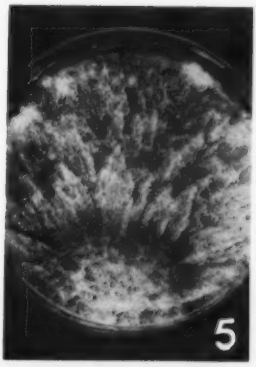
1

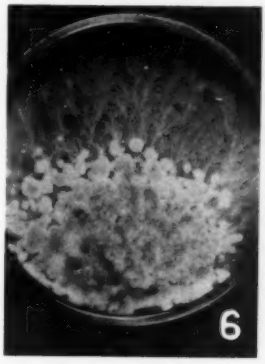


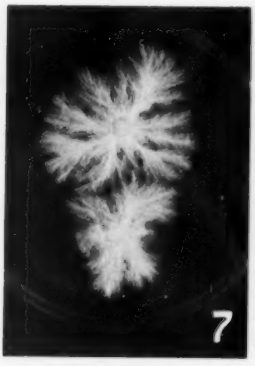
2

3

4


5


6


7

8

'Stag-head' tops, or dead tops, of cedar are a well-known feature associated with this species in all regions, and notably numerous in certain localities. The possible causes of the dead tops have aroused much interest. It would appear that they arise chiefly from some physiological die-back, no pathogens of likely importance having been found associated with the condition. The results of this study to date indicate that dead tops are not an important entrance for decay, and the number of heartwood infections resulting from these tops is too few to consider the defect as a definite indication of decay.

Branch stubs, a common infection court for trunk rots in many species, rarely provide entrance for decay organisms in cedar. A small proportion of the total infections do occur through this avenue, however.

'Dry-side', a defect responsible for the complete cull of a pole tree, is the result of the death of the cambium in a narrow to wide strip down the side of the tree. The cause of 'dry-side' appears to be severe bruising of the cambium tissues by mechanical agencies, such as grazing by a falling tree. Other non-pathogenic factors may be responsible for some of the damage. Except in severe cases the bark usually remains intact over the dead areas and the defect is not diagnosed until the tree is felled. As an avenue for the entrance of decay 'dry-side' was not found to be of importance.

Basal infections through injured roots or means other than wounds were frequent enough, especially in young stands, to warrant further attention than has been given to them in this study. In trees younger than 50 years of age, the well-known root and butt rots, caused by *Poria subacida*, *Armillaria mellea*, *Fomes annosus*, and *Poria Weirii*, have been found to cause appreciable losses in restricted areas, apparently entering through injured roots.

Relative Importance of the Decays

The study of decay in living western red cedar was complicated by two difficulties: first, in many of the infected areas there were two or more organisms, and it was very difficult in most of these cases to separate clearly the damage caused by each fungus; and second, while the study of decay was in progress, only two of the decays could be attributed to known fungi by gross characteristics. It was found necessary and convenient to study the relative importance of the fungi causing decay by grouping them as in Table II.

The incidence of decay in living trees is illustrated in Text-fig. 2. While this graph is fairly reliable for the south-central portion of Vancouver Island, it probably illustrates an incidence too low for the northern Coast and western portions of Vancouver Island. While the age-class distribution for the Interior was poor, the results would indicate a greater incidence of decay in the younger age classes than is found on the Coast.

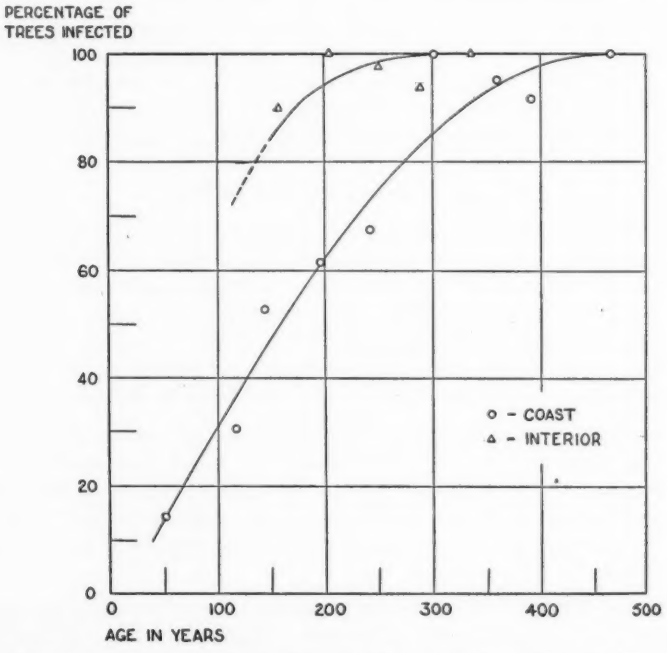
The relative importance of the fungi causing these infections is graphically illustrated in Text-fig. 3. For the purpose of this comparison, all the brown cubical rots not clearly distinguished from that caused by *Poria asiatica* were

TABLE II

THE NUMBER OF TREES INFECTED BY THE PRINCIPAL WOOD-DESTROYING ORGANISMS*

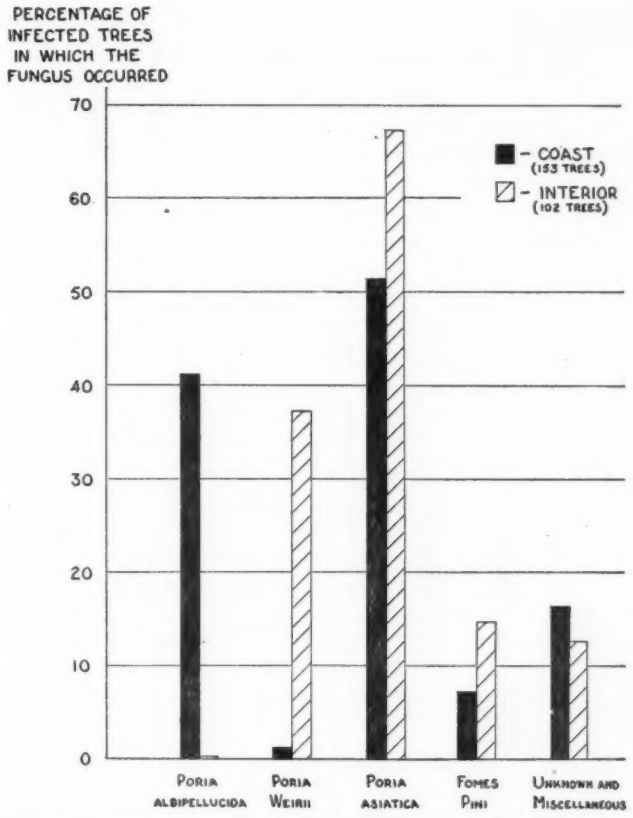
Wood-decaying organism	Interior poles		Coast poles		Coast saw-logs		Total	
	Total no. of infections	No. of mixed infections	Total no. of infections	No. of mixed infections	Total no. of infections	No. of mixed infections	Total no. of infections	No. of mixed infections
Poria Weirii	40	27	0	0	2	0	42	27
Poria asiatica	72	36	79	25	74	37	225	98
Poria albipellucida	1	0	63	21	126	35	190	56
Fomes Pini	16	9	11	4	3	2	30	15
Mixed infections:								
Poria Weirii and Poria asiatica	25	—	0	—	0	—	25	—
Poria albipellucida and Poria asiatica	0	—	21	—	35	—	56	—
Fomes Pini and Poria asiatica	8	—	4	—	2	—	14	—
Inseparable mixtures	5	—	7	—	10	—	22	—

* *One hundred and ten trees were examined in the Interior and 615 trees on the Coast.*



TEXT-FIG. 2. *Relation of the occurrence of decay to age in western red cedar.*

grouped together under this specific name. *Fomes annosus*, *Poria subacida*, and others were placed under "Unknown and Miscellaneous," as their occurrence was too irregular or unimportant to warrant further attention. It will

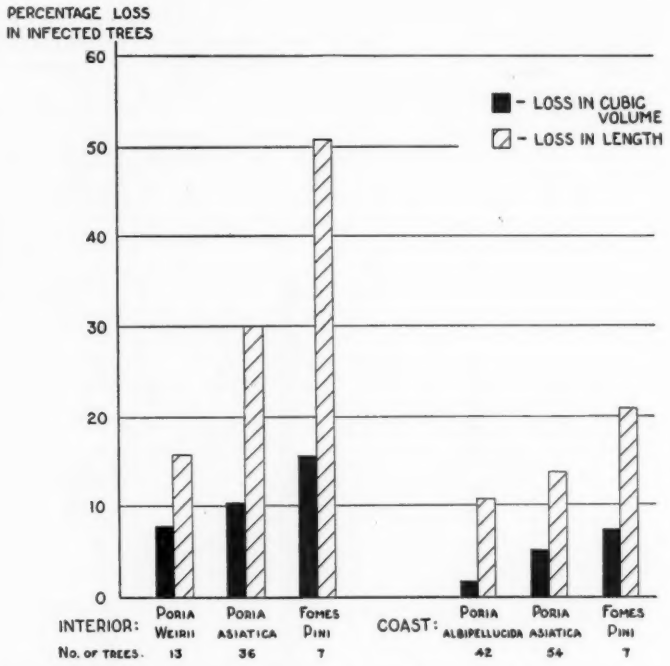


TEXT-FIG. 3. *Relative importance of the wood-destroying fungi expressed as a percentage of the total number of infections in diseased trees.*

be noted that, on adding together the percentage of infections that occurred, the total exceeds 100% for both regions. The reason for this is the number of mixed infections that occurred, and whenever the two or more attacking fungi could be identified, all species involved were given credit for one infection. The infections by causative fungi are outlined in Table II.

While considering the figures presented, it would seem advisable to inspect the relative losses brought about by the fungi in question. In preparing Text-fig. 4, only trees infected by one organism were considered, and the percentage presented is the average loss in these single-infection trees.

Combining the information presented in Text-fig. 3 with that of Text-fig. 4, it is evident that *Poria asiatica* brown cubical decay is the most destructive on cedar throughout all regions, both in total volume of wood destroyed and



TEXT-FIG. 4. *Percentage loss in cubic foot volume and length in infected trees of merchantable pole size by the principal wood-destroying organisms.*

in total lineal loss to the pole-cutter. Of the infected trees studied, nearly 70% were attacked by this fungus in the Interior and over 50% on the Coast (Text-fig. 3). The loss in the cedar of the Interior is somewhat greater than on the Coast, 30% of the length and 10% of the cubic volume of infected, pole-sized trees being lost in the former region, and approximately 15% of the length and 5% of the cubic volume of infected trees being destroyed in the latter region (Text-fig. 4). In several cases *Poria asiatica* has been found attacking small, oppressed cedars from 20 to 50 years of age. In such cases the entire heart of the tree was commonly destroyed (Pl. IV, Fig. 6).

Fomes Pini decay caused considerably more loss than the brown cubical rots (Text-fig. 4), although the incidence of this fungus was low (Text-fig. 3). Of the infected pole timbers studied in the Interior, only 15% were attacked by *F. Pini*. In these infected trees 15% of the cubic volume and 50% of the length of merchantable pole-sized timbers was destroyed. In the Coast

forests, the incidence was not as great, 7.5% of the total infections being initiated by this fungus. The loss was even proportionally lower, 7% of the cubic volume, and 21% of the length of infected, merchantable pole timbers being affected.

The small loss both in length and cubic volume of poles infected by *Poria albipellucida* and *Poria Weirii* indicates that these are essentially butt-rotting organisms, but the high degree of incidence of *P. albipellucida* on the Coast and *P. Weirii* in the Interior is sufficient indication of their importance. *P. albipellucida*, responsible for over 40% of the infections on the Coast (Text-fig. 3), decays less than 2% of the cubic volume, and causes a loss slightly over 10% of the length of infected pole-sized timbers (Text-fig. 4). *P. Weirii* infection in the Interior, although comparatively less frequent than *P. albipellucida* on the Coast, causes a greater loss, approximately 8% of the cubic volume and 18% of the length of infected timbers.

Indications of Decay

No reliable method for predicting the amount of decay in standing western red cedar has been found. The almost complete absence of conks on this species makes the problem of estimating decay very difficult.

As previously discussed, basal wounds are the most important points of entrance for decay fungi. These injuries, however, are of very doubtful value in diagnosing the extent of decay in the heartwood. Although the entire butt may appear decayed, the decay column may have grown up only a short distance past the wounds, while in other cases an apparently sound wound may have provided entrance for a fungus that has completely destroyed the upper merchantable portion of the tree. The number, size, and condition of the basal wounds were thus found to be almost valueless in predicting the amount of decay in a standing tree.

'Stag-head' or dead top and 'dry-side' are not indicative of internal decay. In certain regions where cedar is very decadent trees with these defects offer more possibility of extensive trunk decay. Even in such areas, however, unless a considerable portion of the crown were dead or the 'dry-side' very extensive, it was not common to find decay entering these defects.

Rotten knots and conks, if these can be seen before felling the tree, indicate complete loss of the merchantable bole, unless, as in the case of *Poria Weirii* and *P. albipellucida*, the conks are those of butt-rotting organisms. It is in but a small proportion of the cases where the bole is unmerchantable, however, that either of these indications is present.

In felled trees the discoloration of the wood, particularly in association with the decays caused by *P. albipellucida* and *P. asiatica*, was found to be a fairly good indication of the presence of the fungus, but not of the extent of the decay. After long-butting a pole to remove the decay it was frequently observed that a light core of small diameter, up to 8 in. or more across, was left in the base of the pole. This light brown heart was found to be the

incipient stage of either *P. asiatica* or *P. albipellucida*. In freshly cut logs this is very easily detected in cases of attack by the first fungus, the light heart appearing 'dead' in contrast to the normal brown wood. *P. albipellucida* is not so conspicuous in the incipient stage of decay in a freshly cut log, but as drying takes place it is readily seen, the wood cracking in concentric circles (Pl. I, Figs. 4 and 5).

Up to the present no definite correlation has been found between the diameter of the rot column and its length, or between the species of attacking fungus and the length of the rot column.

Relation of Decay to Age and Vigour of Trees

The normal procedure of plotting periodic growth increment against the decay loss for the same period to determine the pathological rotation of the species, or to find the age at which decay loss exceeds growth increment, showed that at no time during the 450 years of growth covered by this study, did decay increment equal or exceed growth. The limited data upon which these computations were made may have distorted the true results, but it seems probable that such an observation on western red cedar may be true for the age period studied.

In trees of vigorous growth, 35 to 50 years of age, many of the major decay organisms of mature trees were found to cause severe damage. For example, *Poria albipellucida* and *P. Weirii* were found to infect all the heartwood in several cases, a condition not found in older trees. In one area on Vancouver Island where 50 trees averaging 50 years of age were inspected, two out of a total of seven infections were by *Fomes Pini* and both these were through basal wounds. A group of seven 35-year-old cedars at Cowichan Lake, Vancouver Island, were completely rotted by *Poria subacida*. This decay extended up the trees approximately 10 feet.

Another observation of some interest was on the condition of young, oppressed trees, which are of frequent occurrence in most forest stands containing cedar. Although no systematic sampling was carried out, it was found that a large number of the trees that were inspected were badly decayed. *Poria asiatica*, *P. Weirii*, *P. subacida*, *P. albipellucida*, *Fomes Pini*, and a *Merulius*-like fungus all have been cultured from rot in such trees.

Decay of Logs and Slash

All of the fungi causing decay in living cedars are apparently capable of continuing decay in felled trees and logs, while many of these are also capable of decaying slash or primarily live on slash. The greatest damage is probably caused by *Poria Weirii*, *P. albipellucida*, and *P. subacida*. *Fomes pinicola*, *F. annosus*, and *F. applanatus* have been collected fruiting on cedar logs, but the exceedingly limited reports of these three species of *Fomes* on western red cedar would indicate that their occurrence is only of an incidental nature.

According to Murrill (21), *Poria Weirii* "continues the destruction of the heartwood and later of the sapwood after the tree has fallen and may extend along the entire tree, even attacking the bark." Little has been observed concerning the activity of *P. albipellucida* in felled trees and logs of cedar. It has been found, however, that in hewn railroad ties, culled for laminate heart rot caused by this fungus, the organism has continued growth through the tie, and in one case fruited on the lower side. *P. subacida* is frequently found as an active decay organism of all parts of small logs and immature cedar. It is on such logs that the conks of the fungus most frequently occur.

Two fungi, *Polyporus cuneatus* (Murr.) Overh. and *Hymenochaete tabacina* (Sow.) Lév., have been found widely distributed, causing decay of cedar sapwood and slash. Other species, such as *Polyporus abietinus* (Dicks.) Fr., *P. versicolor* (L.) Fr., *P. semipileatus* Peck, *P. hirsutus* (Wulf.) Fr., and *Poria isabellina* (Fr.) Overh., have been found restricted in distribution or of irregular occurrence. Still others, particularly species of Agaricaceae and Thelephoraceae, appear to be only of incidental or rare occurrence on cedar sapwood and slash.

Polyporus cuneatus (4) is the most important single organism responsible for decay of cedar sapwood and slash (7). Throughout all regions investigated this fungus was found fruiting on logs and branches, most frequently inhabiting densely shaded, moist logs, and growing through the bark seams to form the typical imbricate or resupinate conks. It is also found attacking decorticated areas of dead or living trees. *P. cuneatus* causes a white spongy rot of the sapwood of cedar. *Hymenochaete tabacina* is very general in distribution but of much less importance than *Polyporus cuneatus*. This fungus was found chiefly on dead branches and occasionally on larger material. Except as a decay of slash this fungus is of no economic importance.

P. abietinus, *P. semipileatus*, and *P. hirsutus* were found only in the Interior, while *Poria isabellina* and *Polyporus versicolor* were found only on the Coast, but probably occur in the Interior also. *P. abietinus* was frequently found growing on logs and branches from the bark seams in a similar habitat and manner to *P. cuneatus*. *P. semipileatus* occurred less frequently than *P. abietinus* but may cause extensive decay of sapwood in localized areas. *P. hirsutus* was found only on cedar logs still retaining the bark and in much dryer situations than the other sap rot organisms. All these fungi cause white to yellow, spongy to fibrous sap rots. *Poria isabellina* was frequent on decorticated cedar logs, causing a yellowish pitted rot of the sapwood. This fungus was commonly associated with decay directly beneath the charcoal on the surface of burned logs. *Polyporus versicolor* has been found only to a very limited extent during the course of this study.

Little economic importance is attached to the decays of cedar sapwood and slash. Some loss through decay of the sapwood of cedar poles incorrectly stored, however, might warrant further investigation of the causative fungi at some future date.

Cultural Characteristics of the Fungi

Most of the causal organisms of the decays investigated in this study were identified by making isolations of the fungi from incipient decay and comparing the cultures with those of known fungi. It was found that the fungus in a piece of inoculum did not grow on to the media as successfully on malt agar as it did on potato-dextrose agar. After the fungus was established on potato-dextrose agar, however, it was found that when a transfer was made to malt agar the fungus grew as well as on the former medium.

The isolations obtained from the decays were grown on tannic and gallic acid media for grouping and partial identification. Table III gives the reactions and growth of the fungi of importance in this study on acid media

TABLE III

REACTION AND GROWTH OF THE MAJOR WOOD-ROTTING FUNGI OF WESTERN RED CEDAR ON GALLIC AND TANNIC ACID MEDIA AT THE END OF SEVEN DAYS

Fungus	Isolations	On gallic acid medium		On tannic acid medium	
		Reaction*	Growth, mm.	Reaction*	Growth, mm.
White-rot fungi:					
Poria albipellucida	45	++	40–90	+++	0–20
Poria cinerescens	2	++	50–60	+++	0–tr.**
Poria subacida	5	++++	0–14	+++	20–40
Poria Weirii	2	+++	25–35	+++	15–20
Fomes Pini	13	++++	Tr.–10	+++	Tr.–15
Fomes annosus	6	++++	0	++++	10–20
Omphalia campanella	7	++++	0–tr.	+++	0-tr.
Brown-rot fungi:					
Poria asiatica	45	−to+†	10–20	−to+††	0–tr.
Polyporus balsameus	5	−to+†	10–20	−to+††	0–tr.
Merulius "A"	8	—	20–30	—	0–tr.
Merulius "B"	14	—	10–20	—	0–tr.

* − = *Negative, no brown discoloration of agar.*
\+ = *Diffusion zone light to dark brown, formed under inoculum at centre of mat and visible only from underside of dish.*
++ = *Diffusion zone light to dark brown, formed under most of mat but not extending to the margin.*
+++ = *Diffusion zone light to dark brown, extending a short distance beyond the margin of the mat.*
++++ = *Diffusion zone dark brown, opaque, extending considerably beyond the margin of the mat.*
** *Trace of growth only.*
† *Reaction usually negative.*
†† *Reaction usually a slight brown diffusion zone under the inoculum.*

at the end of seven days, following the procedure outlined by Davidson, Campbell, and Blaisdell (11). Using this method, it was not possible to find consistent differential characteristics between *Poria albipellucida* and *P.*

cinerescens (Bres.) Sacc., and between *Poria asiatica* and *Polyporus balsameus*. It was found, also, that the cultures ascribed to *Merulius* sp. could be divided into two types, "*A*" and "*B*," on the basis of their reactions.

Although the description of the conks of *Poria albipellucida* (3) and *P. cinerescens* (23) would readily separate these organisms, it is on rare occasions only that the conks are found, so most identifications must be made from cultures. No way has been found to date to separate the cultures of these two organisms. On tannic and gallic acid media their reactions are similar and very distinctive. On malt agar their growth is rapid, and certain habits of growth would readily distinguish these two organisms from other fungi. In one week the agar in the Petri dish is covered by a thin, white, appressed mat, showing inverted 'V' formations where the mycelium is thickened (Pl. V, Fig. 5). No fruit body of *P. cinerescens* has been found on cedar during this investigation, so all the white laminate decays, from which these distinctive cultures have been isolated, are ascribed to *P. albipellucida*. It is interesting to note, however, that the Department of Agriculture, Ottawa, Mycological Herbarium contains no sporophores of *P. albipellucida* from cedar in British Columbia, but has specimens of *P. cinerescens* from cedar logs from which the remarkably similar cultures to *P. albipellucida* were obtained.

Polyporus balsameus (16) and *Poria asiatica* (22) are readily separated by a comparison of the sporophores and by the colour of the cultures, but the great similarity in the appearance of the decays led to the discovery of some striking similarities in culture. In very young cultures these two species cannot usually be separated at present and even after some time the similarity in growth habits between the cultures of the two organisms is close. The only point of differentiation found to date between the two organisms in culture is that the chlamydospores of *P. asiatica* remain hyaline, while those of *Polyporus balsameus* darken, giving rise to the previously mentioned difference in the colour of cultures. Cultures of the two organisms from the Interior have fruited.

Whether two organisms are involved in the *Merulius* complex or whether the one species shows considerable variation is not certain. On gallic and tannic acid media it is possible to separate the cultures into two forms: *Merulius* "*A*," which grows rapidly across the gallic acid medium with long, feathery, mycelial processes (Pl. V, Figs. 6 and 7), and *Merulius* "*B*," which grows in a soft appressed mat, 'feathering' only slightly at the edges (Pl. V, Fig. 8). Both forms exude numerous droplets of clear fluid that turns yellow with age.

Omphalia campanella has fruited on three occasions in culture, once from the original inoculum on potato-dextrose agar and twice on a medium suggested by Badcock (1). This fungus is easily distinguished in culture as it commonly produces 'aerial rhizomorphs.'

Check List of Fungi Occurring on Cedar

During the course of this investigation, it was found advisable to collect all fungi fruiting on any portion of red cedar. Not only did such a procedure build up a check list of fungi occurring on cedar, but for some of the decays it uncovered the fruiting body of the causal organism, occasionally small and insignificant in character, and thus made the identification of the decay and fungus possible. Such was the case with *Poria albipellucida*. The following check list, Table IV, represents all the Basidiomycetes found on living and dead western red cedar in British Columbia from 1943 to 1945.

TABLE IV

CHECK LIST OF FUNGI COLLECTED ON WESTERN RED CEDAR IN BRITISH COLUMBIA FROM 1943 TO 1945

Species	Locality of collections
Armillaria mellea (Fr.) Quél.	12, 13, 18*
Auricularia auricularis (Gray) Martin	13
Collybia sp.	12
Coniophora betulae (Schum.) Karst.	13
Coniophora puteana (Schum. ex Fr.) Karst.	16
Coniophora suffocata (Peck) Massee	16
Corticium sp.	1, 3, 7, 11
Corticium bicolor Peck	3
Corticium cebennense Bourd.	3
Corticium coronilla Höhn.	3
Corticium livido-caeruleum Karst.	1, 3
Corticium racemosum Burt.	12
Corticium radiosum Fr.	12
Corticium sulphureum (Pers. ex Fr.) Fr.	1
Crepidotus herbarum Peck	17
Flammula decorata Murr.	17
Flammula liquiritiae (Weinm.) Quél.	11
Fomes applanatus (Pers.) Wallr.	3
Fomes annosus (Fr.) Cke.	1, 3, 7, 9
Fomes nigrolimitatus (Romell) Egel.	7, 11
Fomes Pini (Thore) Lloyd and var. *abietis* Karst.	5, 9, 11, 12, 13, 15
Fomes pinicola (Swartz) Cke.	1, 2, 10
Gloeocystidium ochroleucum Bres.	1
Helvella sp.	3
Hypholoma fasiculare (Fr.) Quél.	3
Hypholoma capnoides (Fr.) Quél.	1
Hymenochaete fuliginosa (Pers.) Bres.	13
Hymenochaete tabacina (Sow.) Lév.	1, 3, 5, 12, 13, 18
Lenzites saepiaria (Wulf.) Fr.	16

* *The numbers refer to localities on the map, Text-fig. 1.*

TABLE IV

CHECK LIST OF FUNGI COLLECTED ON WESTERN RED CEDAR IN BRITISH COLUMBIA FROM 1943 TO 1945—*Concluded*

Species	Locality of collections
Marasmius scorodonius Fr.	3
Merulius fugax Fr.	1
Mycena griseiconica Kauffm.	5
Odontia sp.	1, 3, 11
Odontia alutacea (Fr.) B. & G.	1
Odontia alutacea subsp. *floccusa* B. & G.?	16
Odontia aspera (Fr.) Bourd.	9
Odontia lactea Karst.	12, 13
Omphalia campanella (Fr.) Quél.	4, 7, 8, 12, 15, 18
Peniophora sp.	1
Peniophora crassa Burt.	1
Peniophora flavo-ferruginea Karst.	1
Peniophora sanguinea (Fr.) Bres.	1
Peniophora velutina (DC) Cke.?	1
Phlebia mellea Overh.	13
Polyporus abietinus (Dicks.) Fr.	13, 15, 18
Polyporus balsameus Peck	12, 15, 17
Polyporus caesius (Schrad.) Fr.	19
Polyporus cuneatus (Murr.) Overh.	5, 7, 11, 12, 13, 14, 15, 17, 18
Polyporus dichrous Fr.	13, 16, 18
Polyporus elegans (Bull.) Fr.	3, 12
Polyporus hirsutus (Wulf.) Fr.	16, 18
Polyporus immitis Peck	10
Polyporus perennis (L.) Fr.	5
Polyporus Schweinitzii Fr.	5
Polyporus semipileatus Peck	13, 15, 18
Polyporus undosus Peck	1
Polyporus versicolor (L.) Fr.	5
Poria sp.	1, 13
Poria albipellucida Baxter	1, 2, 3, 4, 5, 6, 7, 8, 11, 13
Poria asiatica (Pilát) Overh.	1, 4, 5, 6, 7, 8, 12, 14, 15, 18, 19
Poria candidissima (Schw.) Cke.	1
Poria isabellina (Fr.) Overh.	1, 5, 6
Poria lenis Karst.	1
Poria nigrescens Bres.	11
Poria sericeo-mollis (Romell) Baxter	1
Poria sinuosa (Fr.) Sacc.	11
Poria subacida (Peck) Sacc.	1, 2, 3, 5, 7, 11, 12, 13, 18
Poria Weirii Murr.	1, 3, 4, 8, 9, 10, 12, 13, 15, 17, 18
Psathyrella sp.	11
Schizophyllum commune Fr.	1
Stereum Chailletii Pers.	3
Stereum rugosiusculum B. & C.	3
Stereum sanguinolentum Alb. & Schw.	18
Tomentella sp.	1
Trametes carbonaria (B. & C.) Overh.	1, 16, 19
Trametes mollis (Sommerf.) Fr.	13
Trametes sepium Berk.?	19

Discussion

The problem of determining the causal fungi of the decays of western red cedar was found to be very difficult. This difficulty made it necessary to stress the collection of fungi fruiting on cedar and the inspection of trees from which large specimens of decay were procurable. The collection and study of discoloured material and the restriction of the investigation to areas where data on total age and decay correlations could be collected were not attempted. All investigations were directed solely to decays and causal fungi.

This investigation, because of the foregoing restrictions in the material studied, uncovered little information of value in the management plans for western red cedar. A study of the figures gathered for the loss from decay in cedar would suggest that decay was never a factor of importance affecting the silviculture and management of the species. This is not believed to be true, however. The figures represent the condition of mature cedar trees selected by the logger or pole-cutter as probably containing a merchantable volume of sound wood or a pole. When considering the possibility of managing young cedar stands, the previous remarks concerning the attack of immature trees must be weighed. In these discussions it is seen that young cedars are apparently quite susceptible to attack by several important heart-rotting organisms. Until a careful study is made of the conditions existing in these young trees, no conclusions can be drawn concerning the significance of decay in its effect on the silviculture and management of red cedar. Observational evidence would indicate that decay in young cedars is of importance and should be studied carefully in relation to the management of the species.

Poria albipellucida and *P. Weirii* occur on the Coast and in the Interior but as heart-rotting organisms the former is only important on the Coast and the latter in the Interior. The reason for this is not clear.

The obscure picture of the decay complex on cedar has not been entirely cleared up by this investigation. It is felt, however, that the principal fungi responsible for the decay in mature trees and an indication of their relative importance, have been determined. Much work remains to be done to bring the knowledge of cedar decays and defects to a plane equal to that of other tree species of major economic importance in British Columbia.

Acknowledgments

The author wishes to express his sincere appreciation to Dr. J. S. Boyce, Forest School, Yale University; Dr. H. T. Güssow, retired Dominion Botanist; Dr. J. E. Bier and Messrs. R. E. Foster and P. J. Salisbury, Dominion Laboratory of Forest Pathology, Victoria; Mr. A. W. McCallum and Dr. M. K. Nobles, Division of Botany and Plant Pathology, Ottawa; Dr. L. O. Overholts, Pennsylvania State College; Dr. H. S. Jackson, University of Toronto; and Dr. A. H. Smith, University of Michigan, for their assistance and advice during this investigation.

Men in the logging, pole-cutting, and milling industries co-operated in giving essential, practical advice, and in allowing the author access to their properties at all times. Through the courtesy of the British Columbia Forest Service transportation was made available for carrying on the investigation in the Interior, and advice and direction concerning the location of cutting in cedar were given by all Forest Service officers throughout the Coast and Interior.

References

1. Badcock, E. C. New methods for the cultivation of wood-rotting fungi. Trans. Brit. Mycol. Soc. 25 : 200-205. 1941.
2. Baxter, D. V. Some resupinate polypores from the region of the Great Lakes. VI. Papers Mich. Acad. Sci., Arts Letters, 20 : 273-281. 1935.
3. Baxter, D. V. Some resupinate polypores from the region of the Great Lakes. IX. Papers Mich. Acad. Sci., Arts Letters, 23 : 285-305. 1938.
4. Baxter, D. V. Some resupinate polypores from the region of the Great Lakes. XII. Papers Mich. Acad. Sci., Arts Letters, 26 : 107-121. 1941.
5. Baxter, D. V. Pathology in forest practice. John Wiley and Sons, New York. 1943.
6. Boyce, J. S. The dry-rot of incense cedar. U.S. Dept. Agr. Bull. 871. 1920.
7. Boyce, J. S. Deterioration of wind-thrown timber on the Olympic Peninsula, Washington. U.S. Dept. Agr. Tech. Bull. 104. 1929.
8. Boyce, J. S. Forest pathology. McGraw-Hill Book Co., New York. 1938.
9. British Columbia Department of Lands. Report of the Forest Branch for the year ending December 31, 1943. Dept. Lands, Victoria. 1944.
10. Childs, T. W. Variability of *Polyporus Schweinitzii* in culture. Phytopathology, 27 : 29-50. 1937.
11. Davidson, R. W., Campbell, W. A., and Blaisdell, D. V. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. Agr. Research, 57 : 683-695. 1938.
12. Eades, H. W. and Alexander, J. B. Western red cedar: significance of its heartwood colorations. Can. Forest Service Circ. 41. 1934.
13. Güssow, H. T. and Odell, W. S. Mushrooms and toadstools. Can. Dept. Agr., Div. Botany, Ottawa. 1927.
14. Hubert, E. E. A butt-rot of balsam fir caused by *Polyporus balsameus* Pk. Phytopathology, 19 : 725-732. 1929.
15. Hubert, E. E. Outline of forest pathology. John Wiley and Sons. New York. 1931.
16. Lowe, J. L. The Polyporaceae of New York. N.Y. State Coll. Forestry. Tech. Bull. 60. 1942.
17. McCallum, A. W. Studies in forest pathology. I. Decay in balsam fir (*Abies balsamea*, Mill.). Can. Dept. Agr. Bull. 104 n.s. 1928.
18. Mounce, I., Bier, J. E., and Nobles, M. K. A root-rot of Douglas fir caused by *Poria Weirii*. Can. J. Research, C, 18 : 522-533. 1940.
19. Mulholland, F. D. The forest resources of British Columbia. B.C. Forest Service, Dept. Lands. 1937.
20. Murrill, W. A. Illustrations of fungi—XI. Mycologia, 4 : 163-166. 1912.
21. Murrill, W. A. An enemy of the western red cedar. Mycologia, 6 : 93-94. 1914.
22. Overholts, L. O. New or little known species of *Poria*. Proc. Penn. Acad. Sci. 13 : 121-125. 1939.
23. Overholts, L. O. Polyporaceae of Pennsylvania, the genus *Poria*. Penn. State Coll. School Agr. Tech. Bull. 418. 1942.
24. Spaulding, P. and Hansbrough, J. R. Decay in balsam fir in New England and New York. U.S. Dept. Agr. Tech. Bull. 872. 1944.
25. Weir, J. R. *Polyporus schweinitzii* Fr. on *Thuja plicata*. (Phytopathological notes). Phytopathology, 11 : 176. 1921.

ON STAINING FOR VITAMIN C IN TISSUES[1]

By Jules Tuba[2], George Hunter[3], and John A. Osborne[4]

Abstract

Guinea-pig adrenals were stained for ascorbic acid. In the normal adrenal, staining was found in both cortex and medulla though with greatest density in the zona fasciculata. The stained scorbutic adrenal showed only a few granules.

The mosses *Mnium cuspidatum* and *Mnium affine* contain ascorbic acid in appreciable amounts. Following staining, fine black granules could be seen in both species. The stain intensity of the granules roughly corresponded to their relative ascorbic acid content.

Having satisfactorily established the validity of the staining solution by the above experiments the development of ascorbic acid in the rose hips of *R. acicularis* was followed throughout the summer. There was found to be some association between the ascorbic acid and chloroplasts. With increasing maturity there developed a gradual accumulation of fine granules in the cells of the tissue as well. These granules showed no apparent relationship to any formed elements in the cells. Staining densities roughly parallel ascorbic acid content.

Introduction

Since Szent-Györgyi (8) observed that the suprarenal cortex reduces silver nitrate, many workers have studied the effects on various tissues that are known to contain vitamin C. Silver nitrate is very easily reduced in ammoniacal solution, much less readily in neutral solution, and in acid solution with comparative difficulty. It does not undergo reduction in solutions of organic acids such as malic, tartaric, citric, and malonic, even when boiled (6) unless there be present in addition an oxidizing agent such as potassium permanganate. Leblond (5) subsequently showed that strongly reducing substances such as adrenalin, polyphenols, various sugars, and cysteine have no effect on acid silver nitrate solution. In developing a technique of staining for vitamin C in tissues of the rat, guinea-pig, and mouse, Bourne (1) found that the only substance other than vitamin C capable of giving a black precipitate with acid silver nitrate in the dark is hydrogen sulphide.

The most important evidence for the specificity of acid silver nitrate staining is obtained from scorbutic and non-scorbutic tissues. Investigators, including Leblond (5), Bourne (1), Gough and Zilva (4), Westergaard (10), Giroud and Leblond (3), Giroud (2), have stained a great variety of tissue, including that of the adrenal, testis, kidney, pituitary, and ovary. It was found that the depth of staining decreased progressively with the development of ascorbic acid deficiency.

[1] *Manuscript received June 18, 1946.*
Contribution from the Department of Biochemistry, University of Alberta, Edmonton, Alta.
[2] *Assistant Professor of Biochemistry.*
[3] *Professor of Biochemistry.*
[4] *Research Assistant.*

Experimental

Reagents

The staining solution of Smyth, Bingley, and Hill (7) was used. This is prepared by adding 5 ml. of 5% acetic acid solution to 100 ml. of 5% silver nitrate solution. A 5% solution of sodium hyposulphite was used to remove the unreduced silver nitrate. Analytically pure silver nitrate and sodium hyposulphite were used in the preparation of the above reagents. The staining solution and the hyposulphite solution were prepared daily and, when not in use, they were kept in a refrigerator.

Materials

Our main concern was the determination of the histological distribution of vitamin C in rose hips and these constitute the main materials used. In the development of technique and to obtain evidence for the validity of the staining, we had recourse to the use of other plant materials, including several species of mosses, and, in addition, adrenal glands from normal and scorbutic guinea-pigs.

Methods

Preliminary experiments with iris leaves and green rose hips showed that plant material is more resistant than animal tissue to penetration by acid silver nitrate solution. For example, intact guinea-pig adrenal glands can be immersed in the staining solution, the excess silver nitrate washed out with sodium hyposulphite solution, and then the tissue, after dehydration, can be embedded and sectioned by routine histological methods. The satisfactory result obtained in this way is shown in Fig. 1. However, the acid silver nitrate will not penetrate similar masses of plant tissue, and it was necessary to modify the staining technique to overcome this difficulty.

The moss, *Mnium*, has leaves that are only one cell thick. Hence, these may be readily stained and examined without sectioning. Figs. 3 and 4 show the presence of fine black granules in concentrations that roughly parallel the vitamin C content.

However, sectioning was necessary to determine the distributions of ascorbic acid in rose hips. Sectioning the fresh material and subsequent staining of the individual sections was accompanied by much loss of the vitamin, probably due to leaching, and by the formation of artifacts stained with the silver nitrate.

After considerable trial and error the following method was adopted. The whole fresh rose hip is embedded in paraffin and mounted on a sliding microtome. The block is sectioned until the top third of the hip is cut away. The block is then immediately immersed in the staining solution where it is left, in the dark, at room temperature for 30 min. The block is now washed with sodium hyposulphite solution. Excess moisture is then removed and sectioning is continued. Sufficient penetration of the tissue by the staining solution is obtained in this way to yield several satisfactorily stained sections. Sections are mounted in Farrant's solution for microscopical examination.

The thickness of the sections varied from 15 to 45 μ, directly with the increasing maturity of the rose hips. As the hips increase in size, the tissue cells increase proportionately in diameter. The thickness of the sections is kept greater than the diameter of the largest cells, in order that there should always be present intact cells. Otherwise it is impossible to get continuous sections of the soft, ripe hips in the unfrozen condition. Freezing would not have solved this problem, because there would still have remained the possibility of some of the black, stained material escaping from the sectioned cells.

The staining method here described, with such variable section thickness, can make no claim to quantitative precision in estimating the ascorbic acid content of the tissue. However, this variation is to a large extent compensated for by the uniform and short depth of focus of the microscope, so that the plates do show a fair parallelism between the intensity of staining and the titration values obtained with 2,6-dichlorophenolindophenol for vitamin C in control samples.

Discussion of Results

Adrenal Glands

In confirmation of the silver staining for vitamin C in animal tissues, Figs. 1 and 2, illustrating conditions in the cortex of the adrenals of normal and scorbutic guinea-pigs, may be referred to. The normal adrenal, with a dye titration value of 109 mgm. ascorbic acid per 100 gm., shows the occurrence in considerable frequency of fine black granules. In the scorbutic animal, with less than 24 mgm. ascorbic acid per 100 gm. adrenal, the stained gland shows only a few granules.

The area photographed in Fig. 1 is in the zona fasciculata, immediately below the zona glomerulosa; in Fig. 2, the area is the same with the zona glomerulosa showing on a boundary distorted in mounting.

In the normal adrenal, staining is found in both cortex and medulla, though most abundantly in the zona fasciculata of the cortex.

The concentration of granules into streaks in the upper region of Fig. 1 is not regarded as typical for the zona fasciculata.

That scurvy was well advanced in our scorbutic animal was proved by bone sections that showed extensive haemorrhage in the marrow and characteristic loss of matrix and degeneration of bone cells.

FIG. 1. *Section of normal guinea-pig adrenal gland, 109 mgm. vitamin C/100 gm., through zona fasciculata.* × *320.*

FIG. 2. *Section of scorbutic guinea-pig adrenal gland, less than 24 mgm. vitamin C/100 gm., through zona glomerulosa and fasciculata.* × *320.*

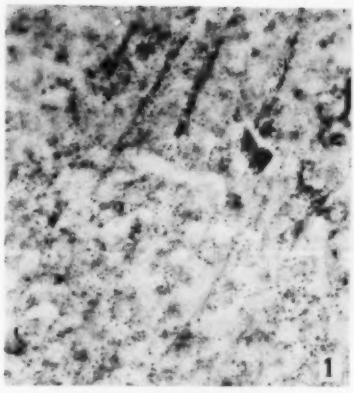
FIG. 3. *Section of Mnium cuspidatum leaf, 33 mgm. vitamin C/100 gm.* × *320.*

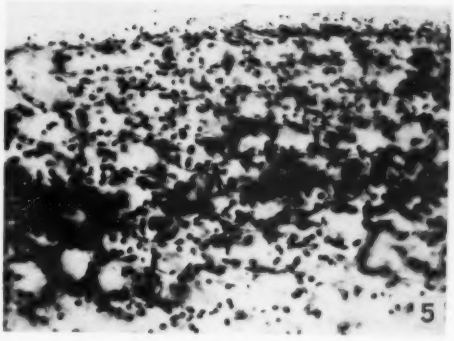
FIG. 4. *Section of Mnium affine leaf, 130 mgm. vitamin C/100 gm.* × *320.*

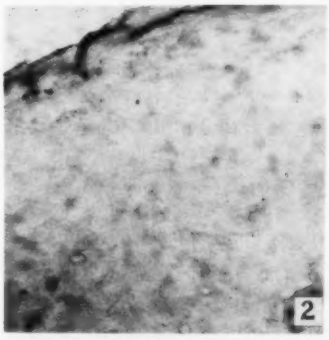
FIG. 5. *Section of rose hip, 140 mgm. vitamin C/100 gm.* × *320.*

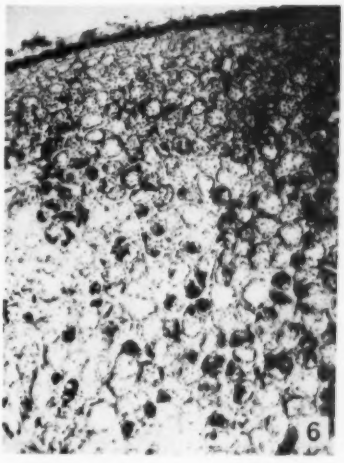
FIG. 6. *Section of rose hip, 300 mgm. vitamin C/100 gm.* × *80.*

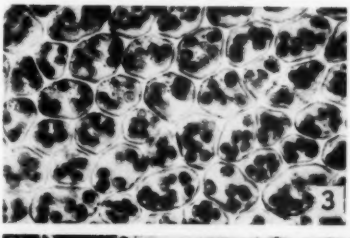
FIG. 7. *Section of rose hip, 300 mgm. vitamin C/100 gm.* × *320.*

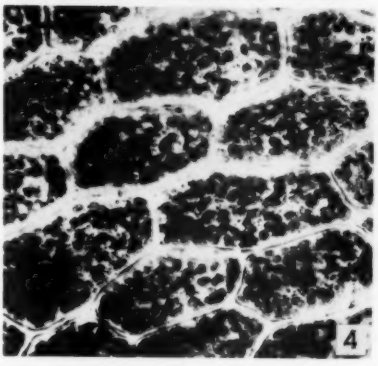

1

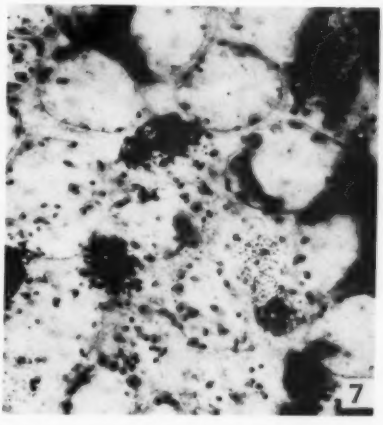

5


2


6


3


4


7

Mosses

The mosses, *Mnium cuspidatum* (L.) Leyss and *Mnium affine* Bland, illustrated in Figs. 3 and 4, are tissues one cell thick but with a marked difference in size of the cells in the two species. Fine black specks can be seen in both species, but more abundantly in *Mnium affine*, which by titration proved to have nearly four times the concentration of ascorbic acid shown by *Mnium cuspidatum*.

The greater size of the cells of *Mnium affine* of course makes strict comparison of the staining in the two species impossible, but the very small depth in the range of focus eliminates to a large extent the differences in the frequency of the black specks that may be due to the different thicknesses of the leaves.

It is evident in both Figs. 3 and 4 that the chloroplasts are not stained. The black specks occur quite distinct from the chloroplasts. Nor is there staining in the intercellular spaces. It would thus seem correct to conclude that vitamin C exists in aggregates in the cytoplasm of these mosses. A search was made in the fresh leaves, with and without cross nicols, for the possible existence of crystals of ascorbic acid, but such were not found.

It may also be noted that no bodies, corresponding to the black specks found after staining with silver, could be found in the fresh leaves, or in those stained with haematoxylin and eosin.

Various other mosses that contained no vitamin by titration were stained. The leaves of most of these were more difficult to examine without sectioning, but in no case where the titration was zero were any black granules discernible.

Table I records our observations with mosses.

It may be noted that at an earlier date we assayed a number of mosses for vitamin C with uniformly negative results (9). This subject is of some interest in relation to vitamin C sources in the Arctic.

Rose Hips

Our observations were confined to *Rosa acicularis* Lindl. The hips were picked throughout the season of development, from the small green preflower stage to the red ripe stage. Table II gives the titration values for material used in Figs. 5 to 12.

Fig. 5 shows conditions at an early stage of development of the rose hip, with small cells, poorly developed cell walls, black stained chloroplasts, and a relatively low ascorbic acid content of 142 mgm./100 gm. of tissue. The round or oval black bodies are certainly chloroplasts that show up in similar sections stained with haematoxylin and eosin. There are few, if any, fine black specks such as were found in the adrenal gland, or in the *Mnium* mosses. It would thus appear that at this stage the vitamin C is definitely associated with the chloroplasts.

At a later stage, Figs. 6 and 7, when the vitamin C has more than doubled its concentration, and the cells have increased in size and definition, there appear besides the black stained chloroplasts, finer black bodies that are not chloroplasts, and that resemble the black specks in the normal adrenal and

Mnium mosses. In both sections there are cells showing marked aggregation of black stained material that appears to be a mixture of stained chloroplasts and non-chloroplastic black granules.

TABLE I

THE VITAMIN C CONTENT OF VARIOUS ALBERTA MOSSES

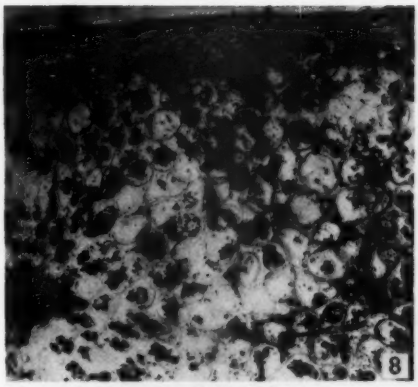
Date	Name of moss	Vitamin C by dye titration, mgm./100 gm.	Result where stained
June 27, 1945	*Mnium affine* Bland	266	+
June 29	*Mnium affine* Bland	254	+
June 30	*Mnium affine* Bland	213	+
June 30	*Mnium affine* Bland	179	+
Aug. 10	*Mnium affine* Bland	133	Fig. 4
July 11	*Mnium cuspidatum*	34	+
Aug. 7	*Mnium cuspidatum*	26	+
Aug. 7	*Mnium cuspidatum*	49	+
Aug. 7	*Mnium cuspidatum*	33	+
Aug. 7	*Mnium cuspidatum*	13	+
Aug. 7	*Mnium cuspidatum*	35	+
Aug. 7	*Mnium cuspidatum*	33	Fig. 3
Aug. 10	*Mnium cuspidatum*	50	+
June 30	*Polytrichum juniperinum*	25	?
June 30	*Funaria hygrometrica*	28	+
June 30	*Aulacomnium palustre*	0	0
June 30	*Sphagnum fuscum*	0	0
June 30	*Sphagnum capillaceum tenellum*	0	0
June 30	*Sphagnum magellanicum*	0	0
June 30	*Sphagnum tenerum*	0	0

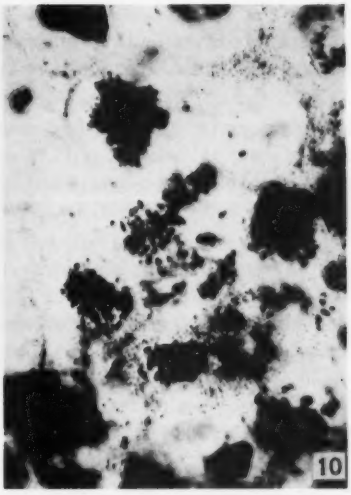
TABLE II

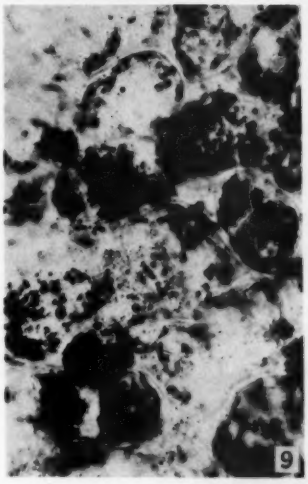
THE VITAMIN C CONTENT IN RELATION TO STAGE OF DEVELOPMENT

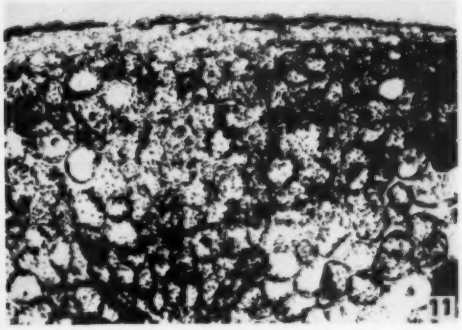
Season	Vitamin C by dye titration, mgm./100 gm.	Stage of development	Figure No.
Spring	142	Preflower, green	5
	305		6, 7
	540		8, 9
Mid summer	848	Postflower, green	
	972		10
	1007		
	1161		
Late summer	1424	Ripe hip	11, 12

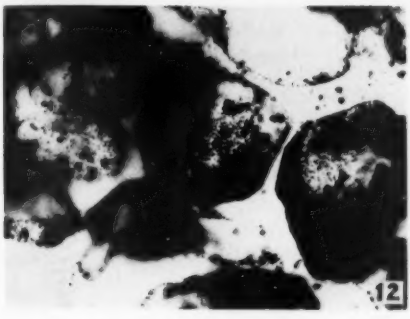
FIG. 8. *Section of rose hip, 540 mgm. vitamin C/100 gm.* × *80.*
FIG. 9. *Section of rose hip, 540 mgm. vitamin C/100 gm.* × *320.*
FIG. 10. *Section of rose hip, 972 mgm. vitamin C/100 gm.* × *320.*
FIG. 11. *Section of rose hip, 1424 mgm. vitamin C/100 gm.* × *80.*
FIG. 12. *Section of rose hip, 1424 mgm. vitamin C/100 gm.* × *320.*


8


10


9


11


12

When the hips are still in the preflower stage but the vitamin C concentration has again almost doubled (Figs. 8 and 9), a comparison of Figs. 6 and 8 shows a more generalized staining corresponding to the increased vitamin C content. The chloroplasts are less regular and the proportion of fine granules has increased.

In Fig. 10, when the flower has been shed, and the hip is green and about three-quarters its mature size and the vitamin C has increased in concentration to 972 mgm./100 gm., the preponderance of fine granules and the formation of dense stained aggregates in the cells are increased in prominence.

Figs. 11 and 12 represent conditions in the ripe stage when the vitamin reaches 1424 mgm./100 gm. The low power picture (Fig. 11) may be compared with the earlier stages (Figs. 8 and 6) to see the increased generalized staining with increased vitamin C concentration. Under the high power the intact cells are seen to be large and only partially in focus. There appear to be some regularly shaped chloroplasts still left but the majority of the chloroplasts appear in process of degeneration, assuming reticular shapes, showing extrusions and extensions that may be aggregates of fine granules. Fine black discrete granules are abundant, as in the central cell of Fig. 12.

Acknowledgments

The authors wish to express their thanks to Dr. R. F. Shaner and Mr. A. G. Fairall, Department of Anatomy, for their constructive criticism of histological technique, and to Dr. E. H. Moss, Department of Botany, for collecting and identifying the mosses, and for information regarding plant tissues.

References

1. BOURNE, G. H. The vitamin C technique as a contribution to cytology. Anat. Record, 66 : 369-383. 1936.
2. GIROUD, A. L'acide ascorbique dans la cellule et les tissus. Gebruder Borntraeger, Berlin. 1938.
3. GIROUD, A. and LEBLOND, C. P. Histological study of renal elimination of ascorbic acid. Anat. Record, 68 : 113-126. 1937.
4. GOUGH, J. and ZILVA, S. S. The silver nitrate staining reaction for ascorbic acid in the adrenal, pituitary and ovary of various species of animals. Biochem. J. 27 : 1279-1286. 1933.
5. LEBLOND, C. P. La vitamine C dans l'organisme. Paris. 1934.
6. MELLOR, J. W. Inorganic and theoretical chemistry. Longmans, Green and Co. Ltd. London. 1923.
7. SMYTH, J. D., BINGLEY, W. J., and HILL, G. R. The distribution of vitamin C in *Nyctotherus cordiformis* Ehrenberg, *Opisthioglyphe ranae* Frölich, and *Toxocara canis* Werner. J. Exptl. Biol. 21 : 13-16. 1945.
8. SZENT-GYÖRGYI, A. Observations on the functions of peroxidase systems and the chemistry of the adrenal cortex. Description of a new carbohydrate derivative. Biochem. J. 22 : 1387-1409. 1928.
9. TUBA, J., HUNTER, G., and KENNEDY, L. L. On sources of vitamin C. II. Alberta native fruits. Can. J. Research, C, 22 : 33-37. 1944.
10. WESTERGAARD, B. Vitamin C in the adrenal glands and the hypophysis cerebri of the ox. Biochem. J. 28 : 1212-1213. 1934.

CULTURAL AND TAXONOMIC STUDIES ON CERTAIN FUSARIA

I. MUTATION IN CULTURE[1]

By J. J. Miller[2]

Abstract

This work represents a continuation of previous studies by the writer on the *Fusarium* of muskmelon wilt, with a view to determining the extent to which the cultural phenomena noted in this organism are characteristic of other Fusaria. The section Elegans is represented by 13 organisms, and the sections Liseola, Discolor, and Gibbosum by one each. Abnormal areas appearing in cultures of these organisms were investigated from the genetic viewpoint, and the results showed the larger of these to be of mutant origin. Evidence was obtained that the 'sclerotia' that appeared in cultures of some of the organisms may likewise be of mutant origin, but this was not deemed conclusive. A comparison of the properties attributed in the literature to 'sporodochia' appearing in Fusarium cultures with those of the patch mutants studied here indicates that the terms are synonymous in this case, and hence the common practice in Fusarium taxonomy of measuring 'macroconidia from sporodochia' is questioned. Since the wild types of the Fusaria studied were readily displaced in culture by mutants, it is evident that some method of maintaining the original isolates in the pure state is fundamental to a consistent taxonomy of the genus. In this connection the practice of maintaining Fusaria in tubes of sterilized soil is recommended, since experiments showed that this type of cultural treatment does not favour the loss of the wild type. A discussion is given concerning the cause of variation and the extent to which it occurs in nature.

Introduction

In previous work on the *Fusarium* of muskmelon wilt already described (23, 24) it was pointed out that initially all the isolates of the organism obtained from plants diseased in naturally infested soil were alike in culture. All produced abundant aerial mycelium on which small non-septate conidia were borne rather sparsely. This cultural form was termed the 'wild type.' When grown in culture the wild type was found to be readily displaced by mutants, and this process could be favoured by transferring the cultures successively to fresh slants. Dense structures appeared frequently in cultures of the wild type. Since monosporous cultures obtained from such areas consistently included a high proportion that were mutant, they were termed 'patch mutants.'

The present studies involve a continuation of this work with a view to determining whether the cultural behaviour observed is also shown by other members of the genus.

Materials and Methods

The 16 Fusaria employed during the present studies were obtained from various sources, as outlined in Table I. Material was received either as freshly isolated cultures or as diseased host material from which isolations

[1] *Manuscript received May 9, 1946.*

Contribution No. 870, Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, Canada.

[2] *Agricultural Assistant, Dominion Laboratory of Plant Pathology, Harrow, Ont.*

TABLE I

SUMMARY OF THE CLASSIFICATION AND ORIGINS OF THE *Fusarium* ISOLATES STUDIED

Organism	Section of genus	Host	Place of origin	Date of isolation	Contributor
Muskmelon wilt *Fusarium**	Elegans	*Cucumis Melo* L.	Ontario	1942, 1943 1944, 1945	
Pea root rot *Fusarium*†	Elegans	*Pisum sativum* L.	Ontario	1945	R. G. Atkinson
Soybean blight *Fusarium**	Elegans	*Glycine max* Merr.	Ontario	1944	
Tomato wilt *Fusarium**	Elegans	*Lycopersicon esculentum* Mill.	Ontario	1944	
Aster wilt *Fusarium*	Elegans	*Callistephus* sp.	Ontario	1944	
Navy bean root rot *Fusarium**†	Elegans	*Phaseolus vulgaris* L.	Ontario	1944	
Gladiolus vascular *Fusarium*	Elegans	*Gladiolus* sp.	Ontario	1945	D. L. Bailey
Cabbage wilt *Fusarium*	Elegans	*Brassica oleracea* L.	Ontario	1945	D. L. Bailey
Flax wilt *Fusarium*	Elegans	*Linum usitatissimum* L.	Ontario	1944, 1945	K. R. Hillier
Cotton wilt *Fusarium*	Elegans	*Gossypium* sp.	Texas	1944	G. E. Altstatt
Cowpea wilt *Fusarium*	Elegans	*Vigna sinensis* Endl.	Texas	1944	G. E. Altstatt
Soil Fusarium *A*	Elegans		Ontario	1945	
Soil Fusarium *B*	Elegans		Ontario	1945	
Fusarium moniliforme Sheldon*	Liseola	*Zea mays* L.	Ontario	1945	
Gibberella Saubinetii (Mont.) Sacc.	Discolor	*Triticum aestivum* L.	Ontario	1945	
Fusarium Scirpi Lamb. & Fautr.††	Gibbosum	*Glycine max* Merr.	Ontario	1945	

* *Pathogenicity confirmed by infection experiments.*

† *On the basis of host relationship and symptomatology these Fusaria would appear to be closely similar to two pathogens described by Snyder (33) as belonging to the section Martiella. However, they were found here to be morphologically so similar to the Elegans Fusaria under study that there seems no question that they belong in this section. The aster and gladiolus Fusaria, for instance, were found to be morphologically more similar to the navy bean* Fusarium *than to the muskmelon or cabbage Fusaria.*

†† *It is not believed that this organism, found fruiting on the root of a diseased soybean plant, was growing as a parasite since the unhealthy condition of the plant appeared due to other causes.*

were made by the writer. Two Fusaria of the Elegans type, presumably saprophytes, were isolated from soil by the plate method and for convenience are referred to as Soil Fusarium *A* and Soil Fusarium *B*. No specific names have been attached to any of the Elegans Fusaria studied. Although it may seem advisable to designate the organisms by some commonly employed system of nomenclature, in view of the conclusions reached in this series concerning the taxonomic situation in the genus, neither the monograph of Wollenweber and Reinking (43) nor the recent revision of the Elegans Fusaria proposed by Snyder and Hansen (34) can be regarded as satisfactory. As will be apparent from a publication to follow, the 'normal' conidium technique upon which the Wollenweber and Reinking monograph is mainly based precludes the identification of the Elegans Fusaria studied here with any of those described in "Die Fusarien." The Snyder and Hansen revision disregards morphological criteria and places primary emphasis on the host relationship. This is not in agreement with the conclusion reached during

the present studies, that if proper cultural methods are employed the morphology of these organisms will be found sufficiently constant to warrant an attempt at morphological classification. Hence it is felt that the practice adopted in Table I of listing the host and the type of disease caused by a given organism cannot be improved upon at the present time. In the case of the last three Fusaria listed in the table, the nomenclature of "Die Fusarien" has been followed although, except in the case of *F. moniliforme*, it was not found possible to reach a satisfactory determination on morphological grounds. *Gibberella Saubinetii* was identified as such mainly on the basis of host relationship and symptomatology. The organism designated as *F. Scirpi*, owing to the elongate terminal cells of the conidia it produced on the soybean plant, apparently belongs in Wollenweber's section Gibbosum, and it was found to fit more closely the description of the above-mentioned organism than that of any other given in the section. It should be pointed out that the pathogenicity of all the organisms studied has not been confirmed by infection experiments and the possibility exists that some of these were saprophytes and were not attacking the host in question. This would not be a serious drawback as far as the present studies are concerned since these organisms were employed mainly to determine whether other Fusaria display a cultural behaviour similar to that of the muskmelon pathogen.

Stock cultures of the organisms were prepared in the following manner: shortly after isolation each organism was purified by the single spore technique and tubes of moist sandy loam soil (sterilized) were inoculated from young single spore cultures of each. The contents of these tubes slowly became dry and soil grains from them were employed during subsequent experiments as a source of inoculum, a practice that was justified by the results of an experiment on cultural degradation to be described.

The technique followed for isolating single spores has been outlined previously (23). The nutrient medium most frequently employed was potato dextrose agar prepared according to the formula of Riker and Riker (28). Soil agar (or, more correctly, soil infusion agar) was prepared from sandy loam soil according to the method previously described (23).

The nomenclature employed here for the mutants is that of the writer's preceding publications (23, 24). It consists of a binomial system in which the first word (light, intermediate, dark) refers to the depth of pigmentation of the mycelium immersed in the agar substratum, and the second (raised, semi-raised, appressed) to the amount of aerial mycelium present. The wild type of each *Fusarium* studied was designated as "light raised" and provided a standard of comparison for the mutants derived from it.

For spore measurements and microphotographs the material under study was mounted in a weak aqueous solution of ruthenium red, following the method employed by Brown and Horne (9).

Monosporous Isolations from Abnormal Areas

All the Fusaria studied here, when grown in culture on potato dextrose agar, produced a loose mycelial type of growth that spread out uniformly from the point of inoculation and bore small conidia evenly and in most cases abundantly with no evidence of localization. In the Elegans Fusaria the uniformity of the mycelial growth was commonly disturbed by two main types of cultural phenomena to which the terms 'patch mutant' and 'sclerotium' have been variously applied. These will be considered in turn.

(A) *Patch Mutants*

In the present studies the purpose of the first part of the experimental work was to determine whether areas similar in appearance and in origin to the patch mutants of the muskmelon *Fusarium* occurred in other members of the genus. In this connection Petri dishes poured with 15 cc. of potato dextrose agar as well as test tube slants prepared with the same medium were inoculated from the soil tube stock cultures or with single spores of the organisms in question. The resulting growth in each case was carefully scrutinized for the appearance of abnormal areas in the otherwise uniform mycelium. These sometimes appeared early, becoming evident within three or four days in cultures originating from a single spore, but usually were not conspicuous until a few weeks had elapsed. Monosporous isolations were made from a large number of such places, and also from adjacent normal-appearing areas, which served as checks, about 20 single spore cultures being obtained in each case. In Table II the results of this work are summarized. It will be noted that in all the organisms tested, a large proportion of the cultures thus obtained from the patch mutants were of a type that differed from the 'parent' form. A total of 86 patch mutants were investigated in this manner, and from 66 of these, mutants were obtained. It is likely that more would have yielded mutants had a greater number of single spore cultures been obtained. In all but four instances only one type of mutant was obtained from a given patch mutant, thereby indicating that each of these had resulted from a single mutation. The patch mutants concerned in the four cases mentioned each yielded two mutant types. Although mutants were obtained in a few cases from normal-appearing areas, the number was comparatively low. The indication is, however, that mutant material is not necessarily confined to visibly abnormal areas of the cultures.

The patch mutants encountered during these studies showed a wide range of morphological variation. The commonest type throughout the series was expressed as a loose, rather diffuse area that first became evident in the aerial mycelium as shown in Fig. 1 (A), and tended to spread until a fairly wide area was covered, as in Fig. 9 (F). Both these illustrations are from the muskmelon *Fusarium*, and two other examples from this organism are shown in Fig. 6. A number of such patch mutants appearing in a slant culture of *F. moniliforme* are shown in Fig. 4. In the latter the patch mutants were rendered very conspicuous by the fact that the aerial mycelium collapsed

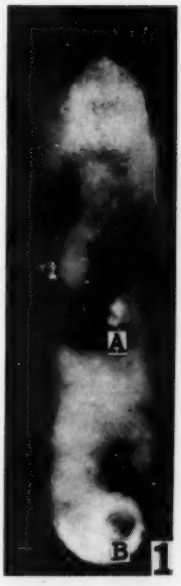
TABLE II

SUMMARY OF THE RESULTS OBTAINED BY MAKING MONOSPOROUS OR MONOHYPHAL ISOLATIONS FROM ABNORMAL AREAS APPEARING IN CULTURES OF *Fusarium*

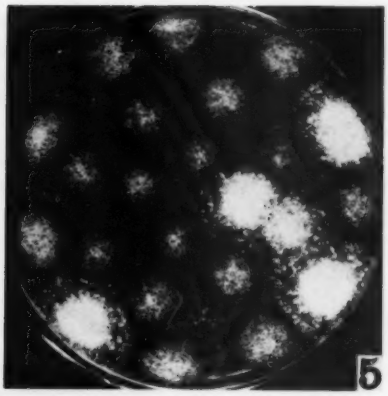
Organism	Patch mutants		Sclerotia		Normal-appearing areas	
	Number tested	% of isolates that were mutants	Number tested	% of isolates that were mutants	Number tested	% of isolates that were mutants
Muskmelon *Fusarium*	19	46	6	0	11	1.5
Pea *Fusarium*	6	37	13	1.5	6	0
Soybean *Fusarium*	10	10	20	0	17	0.4
Tomato *Fusarium*	5	54	—	—	3	0
Aster *Fusarium*	3	11	—	—	1	0
Navy bean *Fusarium*	6	19	—	—	4	0
Gladiolus *Fusarium*	6	19	—	—	2	0
Cabbage *Fusarium*	9	21	—	—	4	1.1
Flax *Fusarium*	4	41	—	—	4	1.4
Cotton *Fusarium*	2	39	—	—	2	0
Cowpea *Fusarium*	2	45	—	—	1	0
Soil Fusarium *A*	5	60	4	0	4	0
Soil Fusarium *B*	4	49	—	—	2	0
F. moniliforme	2	52	—	—	1	0
Gibberella Saubinetii	2	77	—	—	1	0
F. Scirpi	1	100	—	—	1	0
Totals	86		43		64	

elsewhere in the culture. Fig. 5 shows the result of making monosporous isolations from the largest of the patch mutants in Fig. 4. It will be noted that of the 23 cultures on the plate, five are mutants with abundant aerial mycelium. Similar isolations from the appressed area of the slant shown in Fig. 4 yielded all wild type cultures. In Fig. 7 is shown a patch mutant of the flax *Fusarium* photographed from beneath; a distinct darkening effect is noted, which is rather unusual, since most of the loose type in this organism as well as in others had no marked effect on pigmentation. The result of making monosporous isolations from this patch mutant is shown in

FIG. 1. *Slant culture of the muskmelon* Fusarium *inoculated 21 days previously, showing two patch mutants (A and B).* FIG. 2. *Appressed mutant culture of muskmelon* Fusarium *obtained by transferring four successive times a culture similar to that shown in Fig. 1.* FIG. 3. *Large patch mutant and a number of smaller ones appearing in a month-old slant culture of Soil Fusarium A.* FIG. 4. *Numerous patch mutants in a month-old slant culture of* F. moniliforme *Sheldon.* FIG. 5. *Plate of monosporous cultures obtained from the large patch mutant near centre of slant culture shown in Fig. 4. The five white cultures are mutant and the remainder wild type.* FIG. 6. *Two patch mutants appearing in a Petri dish culture of the muskmelon* Fusarium. FIG. 7. *Patch mutant in similar culture of the flax* Fusarium, *as viewed from below.* FIG. 8. *Twenty-day-old monosporous cultures of the muskmelon* Fusarium *showing four plectenchymatic patch mutants (A, B, C, D) in an early stage of development.* FIG. 9. *The cultures in Fig. 8 photographed 25 days later, showing advanced stage of development. During the interim two patch mutants (E, F) have become evident.* FIG. 10. *Same as Fig. 9 but photographed by transmitted light. The dark area to the right of D indicates presence of another patch mutant not visible in Fig. 9.*

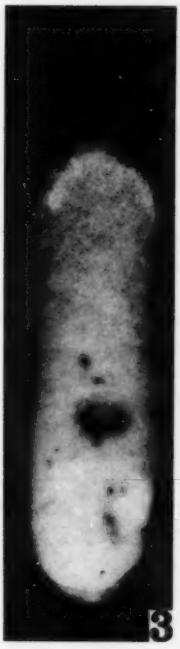

A
B
1

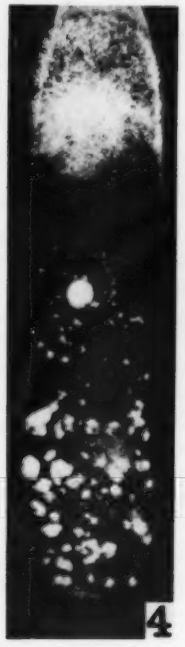
2

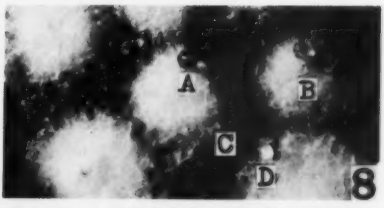

5

6

7


3


4


A
B
C
D
8

F
A
B
C
E
D
9

F
A
B
C
E
D
10

Fig. 22. Loose mound-like patch mutants were sometimes so numerous in Petri dish cultures of the flax *Fusarium* that they made contact with each other all over the surface, concealing the mycelium of the parent. A somewhat similar effect was noted with *Gibberella Saubinetii*, but here the patch mutants were not nearly as abundant. They were likewise prominent in the pea *Fusarium*, and tended to be much larger, one being noted that had achieved a diameter of 10 mm. The tomato *Fusarium* provided another interesting variation of this type of patch mutant, since, although fairly large (up to 5 mm. in diameter) they tended to be very inconspicuous. Transmitted light was helpful in locating these since the patch mutants were denser than the surrounding culture.

The other main type of patch mutant was characterized by the presence of a certain amount of 'plectenchyma,' composed of swollen mycelial cells with thick walls. Razor sections of plectenchyma had a distinctly cellular appearance and the term "Plectenchyme" applied by Appel and Wollenweber (2) to similar structures that they found in cultures of *Fusarium* seems appropriate. Patch mutants containing plectenchyma may be separated into two groups: (1) those in which a dense growth of aerial mycelium is associated with the plectenchyma, and (2) those composed almost exclusively of plectenchyma. The first group has been observed to appear consistently in cultures of the muskmelon *Fusarium*. Figs. 1 (B) and 9 (E) provide examples. These patch mutants tend to be deeply pigmented as viewed from beneath, and this dark appearance usually becomes evident from above later on, showing through the aerial mycelium, which eventually disappears over the area. On occasion the darkening effect may be almost completely absent and the structures assume a light brownish colour. Among the other Fusaria studied, the patch mutants of the cabbage pathogen resembled this type rather closely, but the darkening effect did not occur. The other organisms rarely developed this type of patch mutant, but an example is shown in Fig. 3. Here, however, plectenchyma development was slight. The second group mentioned above was widespread among the Elegans Fusaria, only the pea, cabbage, and flax Fusaria failing to develop them conspicuously, and they were especially prominent and abundant in the muskmelon, aster, navy bean, and gladiolus Fusaria. These bodies first become evident as droplets of clear liquid resting on the aerial mycelium, as shown in Fig. 8. It would perhaps be more correct to regard the droplets as hanging downward from the aerial mycelium since the Petri dishes were always kept inverted. About a week later a light brownish body is noted beneath the drop and it gradually increases in size, attaining a diameter up to 4 mm. or more. This development is illustrated in Figs. 8 and 9, the time interval being 25 days, during which the bodies became greatly enlarged and the drops finally disappeared. It is apparent from Fig. 10 in which transmitted light was employed that they are dense in structure and are sharply distinct from the surrounding mycelium. The body labelled C in Fig. 10 presents the appearance of spreading from a central point of origin. Razor sections of these bodies examined micro-

scopically revealed them to consist of a thick rind-like wall about 100 to 300μ in thickness enclosing a central hollow usually containing some loose hyphal material. These features are illustrated in Fig. 18, which shows a longitudinal section of one of the bodies from a culture of the gladiolus *Fusarium*. Knotted or bladder-shaped bodies possessing a tissue-like or cellular structure have been described in cultures of *Fusarium* by Appel and Wollenweber (2), and these would seem to correspond to this type of patch mutant.

An unusual type of patch mutant that cannot be grouped with any of the preceding was noted in *F. Scirpi*. It took the form of a soft, mound-like body about 3 mm. in diameter that was found to be composed almost entirely of macroconidia, with no plectenchyma and very little mycelium.

In addition to the differences in general appearance and in compactness, the patch mutants frequently differed from the surrounding normal areas of the cultures in regard to type and abundance of sporulation. Chlamydospores were usually very abundant in patch mutants; in fact in the tomato *Fusarium* the presence of these spores was an aid in locating the relatively inconspicuous patch mutants found in this organism. Certain of the patch mutants were found to produce large numbers of long, multiseptate spores.* This kind of patch mutant was definitely in the minority, comprising not more than 10 to 20% of the total, and these showed no consistent morphological differences from those that bore few or no macroconidia. For example, although only the larger of the two patch mutants shown in Fig. 6 bore macroconidia, there was no obvious morphological difference between them other than size, which elsewhere was not found correlated with macroconidial production.

Efforts to correlate the types of patch mutant with the types of mutant they yielded were not consistently successful. In Fig. 1, for instance, although the two patch mutants are quite dissimilar in appearance, the same type of mutant (light appressed) was obtained from each. The two patch mutants shown in Fig. 6 appear to be of the same general type but the mutants isolated from them differed. A dark semiraised mutant was obtained from the larger one and a light raised mutant from the other. In Fig. 9 some evidence of correlation was noted since (E) yielded light appressed mutant cultures and (F) light semiraised. The mutants obtained from the patch mutants that consisted almost entirely of plectenchyma were mostly of the light raised type, closely resembling the wild type.

In one respect, however, a definite correlation was established between the nature of the patch mutants and that of the mutant cultures isolated from them. Monosporous isolations from patch mutants producing abundant

** The terms 'macrospore' and 'macroconidium' are commonly applied to Fusarium spores of this type in the literature to distinguish them from the smaller nonseptate spores or 'microconidia.' These terms will be employed here with the proviso that the two types of conidia are not regarded as sharply distinct from each other, a view that is in conformity with the opinion expressed by Appel and Wollenweber (2). Macroconidia would appear to be simply large microconidia and this is supported by the observation that in mutants that produce both macro- and microconidia it is usually possible to find all gradations of size and shape between the two extremes. The same is true of the wild types under conditions in which they produce macroconidia as well as microconidia.*

macroconidia usually yielded mutants that likewise produced abundant macroconidia. But in a few exceptional instances the mutants obtained from patch mutants producing a small proportion of macroconidia (up to 10%) failed to produce anything but microconidia when grown in the pure state on the same medium. Since the wild types also produced only microconidia, it is evident that in some manner the mixture of the two strains led to the production of macroconidia. Chlamydospores, as stated above, were generally abundant in the patch mutants, and most of the mutants obtained from the latter produced this type of spore more abundantly than did the wild type. It should be noted here that the quantity of chlamydospores and the proportion of macroconidia to microconidia tended to increase as the cultures aged.

Since patch mutants are thus shown to be quite common in cultures of a variety of Fusaria, one would expect to find structures of similar origin mentioned frequently in the voluminous literature on the genus. This, however, is not the case. References to 'sporodochia,' on the other hand, are very numerous, but in the cultures studied here no structures were ever observed that, in the writer's opinion, could properly be termed sporodochia. These two prominent discrepancies could readily be resolved were it established that the 'sporodochia' are actually patch mutants. In this connection it may be pointed out that the patch mutants bear a strong similarity to the 'sporodochia' of previous writers in two important respects: (*a*) in morphology, and (*b*) in cultural behaviour. To consider the former, Appel and Wollenweber (2) state in regard to sporodochia: "Wo Sporodochien entstehen, können diese aus einem lockeren fädigen Hyphengeflecht hervorkommen oder aus mehr oder weniger begrenzten Plectenchymknäueln." These two types of sporodochia correspond very closely to the two main types of patch mutants described above. Sherbakoff (30) regards Fusarium sporodochia as being with or without a plectenchymatic base and without any differentiated enclosure. This is also true of the patch mutants. Fusarium sporodochia are regarded as a useful source of macroconidia for taxonomic purposes. As Wollenweber *et al.* (44) state: "If we realize the fact that tubercular sporodochia with normal and uniform conidia occur in the majority of Fusaria that can be easily grown in pure culture, there will be no difficulty in judging the normal stages and, consequently, in identifying most of these fungi." In the present studies macroconidia were often found to be very abundant in patch mutants. Regarding (*b*) above, transfers from patch mutants were found, as expected, to give rise to mixed cultures of parent and mutant in which the mutant had a strong tendency to predominate. Certain statements by other writers give clear indication of similar genetic differences between the 'sporodochia' and the surrounding mycelium. In speaking of the plectenchymatic structures that they regard as a form of sporodochium, Appel and Wollenweber (2) state: "Eigentümlich war die Tatsache, dass die Plectenchymentwicklung ebenso wie die der Konidien und des Mycels potenziert werden konnte, wenn man Teile derselben Wuchsform impfte." Wollenweber *et al.* (44) recommend that for the production of abundant spores, transfers should be made from

"sporodochia or pionnotes." Sherbakoff (30), in his description of *F. sclerotioides* makes the following statement: "Cultures of this organism on all media may for a long time produce microconidia almost exclusively; but if mature macroconidia from occasionally produced sporodochia are planted, macroconidia may be produced again in new cultures in great abundance and on almost any medium." More recently McLauchlin (22) in regard to a *Fusarium* attacking *Cereus schottii* states: "In the first cultures examined, non-septate spores were produced almost exclusively on all media. When septate spores were transferred from sporodochia, which occurred occasionally, cultures producing septate spores were obtained." There thus seems little doubt that the 'sporodochia' described by these authors as occurring in cultures of *Fusarium* were actually patch mutants.

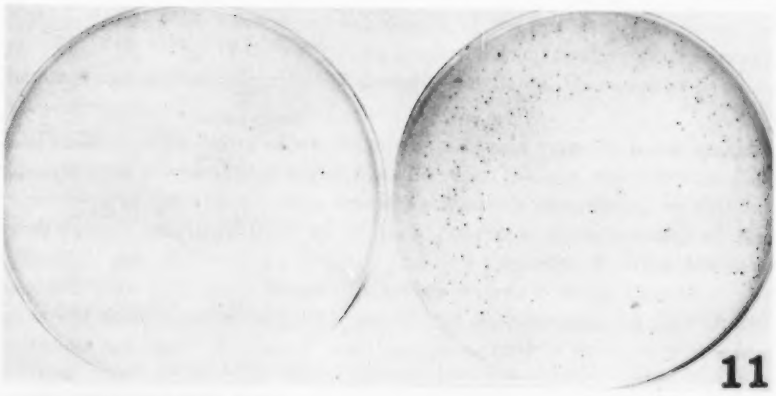
The importance of this conclusion to Fusarium taxonomy is obvious since measurements of "macroconidia from sporodochia" are frequently encountered in descriptions of species of *Fusarium*. If these structures are the result of mutations occurring in the cultures studied, then it is reasonable to question whether such measurements can be of reliable diagnostic value. Since a given patch mutant may have resulted from the appearance of any one of a great variety of mutants, it seems over-optimistic to hope that conidia obtained from such structures would be sufficiently constant to serve for identification. This point will be considered more fully in the second paper of the present series.

(B) *Sclerotia*

In descriptions of Elegans Fusaria references to dark blue or bluish-green sclerotia are often encountered. In the present studies structures corresponding to these appeared frequently in cultures of the muskmelon, pea, and soybean Fusaria, and in Soil Fusarium *A*, but were very few or absent in the others. Sometimes these were very abundant, as many as 200 or more appearing in a single Petri dish culture, and they differed from any of the patch mutants already described in that, as a rule, they were much smaller (1 mm. in diameter or less) and were dark bluish-green in colour. In the earlier stages of development they were usually light brown. In common with the plectenchymatic type of patch mutant illustrated in Fig. 18, the sclerotia were found to consist of a dense wall surrounding a less compact central region,

---

FIG. 11. *Induced sclerotia in pea* Fusarium, *as viewed through bottom of Petri dish. Left, check plate inoculated with a spore suspension of the wild type before the agar solidified. Right, same as check but conidia and mycelium of the mutant shown in Fig. 12 were added to the wild type spore suspension before inoculating. Photograph taken 20 days later.* FIG. 12. *Six-day-old slant cultures of wild type (right) and mutant isolated from sclerotium of the pea* Fusarium. FIGS. 13 and 14. *Razor sections of two sclerotia from 37-day-old single spore cultures of the pea* Fusarium. ×75. FIG. 15. *More detailed view of central region of the section shown in Fig. 14, indicating cellular structure.* ×330. FIGS. 16 and 17. *Razor sections of two induced sclerotia from 15-day-old mixed culture prepared at same time and in same manner as that shown in Fig. 11.* ×75. FIG. 18. *Longitudinal section of plectenchymatic patch mutant of gladiolus* Fusarium *from 15-day-old single spore culture.* ×75.

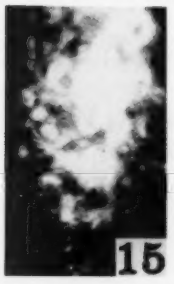

11

12

13

14

16


15

17

18

and these features are evident in the sclerotia of the pea *Fusarium* shown in Figs. 13 and 14. Fig. 15 shows the plectenchymatic structure in more detail, indicating that the bodies consist of densely compacted, thick-walled hyphae of varying width.

The structural similarity of the sclerotia to certain types of patch mutant suggested that a connection might likewise exist between the sclerotia and the incidence of mutations. This was investigated in some detail by making 'biscuit-cutter' isolations from 43 of these, obtained from cultures of the muskmelon, pea, and soybean Fusaria, and Soil Fusarium *A*. The sclerotia concerned were crushed in drops of distilled water on slides to obtain suspensions of conidia and mycelial fragments that were streaked on agar plates. The results are listed in Table II and, as shown, only in the case of the pea *Fusarium* were mutants obtained. From two sclerotia produced by this organism slow-growing mutants were isolated. One of these consisted mainly of immersed brownish mycelium that produced extremely abundant macroconidia and these covered the cultures in a thick slimy layer. This mutant is illustrated in Fig. 12. It was found possible to reproduce sclerotia experimentally by preparing spore suspensions of the mutant and of the wild type and mixing a few drops of each in Petri dishes containing 15 cc. of potato dextrose agar just before the latter solidified. About 10 days later sclerotia began to appear in the plates containing the mixture whereas in plates containing the wild type alone they did not become evident until at least two weeks after inoculation and even then were much less abundant. The results of this experiment are illustrated in Fig. 11. Razor sections of the 'induced' sclerotia are shown in Figs. 16 and 17, and it is apparent that structurally these are quite similar to those in Figs. 13 and 14, which appeared spontaneously in wild type cultures.

The foregoing is strong evidence for the view that these sclerotia are of mutant origin. However, the failure to obtain mutants from the many other sclerotia studied in this manner vitiates the conclusion to some extent. If Fusarium sclerotia always result from mutations, it should be possible to isolate mutant material from them more readily. Frequently the point of origin of a sclerotium is seen to be a small piece of debris in the agar. Possibly, therefore, a stimulus is required to initiate sclerotial development, and mutation may represent one such stimulus.

Since the Fusaria are commonly regarded as imperfect stages of Ascomycetes, the general appearance of the razor sections could be taken as suggesting that the sclerotia are actually abortive perithecia. No evidence of asci or ascospores was found in the crushed mounts and sections that were studied.

It is not implied that sclerotia, and likewise sporodochia, wherever they appear, may be of mutant origin. It must be emphasized at this point that reference is made here only to structures arising in cultures of *Fusarium*.

Cultural Degradation

In a preceding publication (23) the phenomenon termed 'cultural degradation' was discussed. The term was employed to designate the process by which the wild type of the muskmelon *Fusarium* was displaced in culture by mutants. An example is provided by Figs. 1 and 2. The former shows a slant inoculated with a purified culture of the wild type and the latter a similar culture that was transferred four times during a period of three months subsequent to inoculation, as a result of which the original 'raised' character of the culture was lost and the immersed mycelium of an appressed mutant type became predominant, as illustrated.* The term 'deterioration' has been applied by Lucas (20) to a similar phenomenon in *Colletotrichum falcatum* Went. in which "patch variants" appeared and tended to displace the original type. The experimental work described previously (23) indicated that the process in the muskmelon *Fusarium* is accelerated by frequent transferring, certain media favouring the loss of the wild type more than others. In tubes of sterilized soil and on slants of soil agar, even when the latter were transferred, the wild type remained pure. The evidence suggested that this was not because the mutants were unable to displace the wild type on this medium but rather that under such conditions of culture the organism was genetically very stable.

To determine whether this situation is more widespread in the genus *Fusarium*, an experiment was performed involving 10 Fusaria under four cultural conditions: (1) potato dextrose agar slants, transferred; (2) soil agar slants, transferred; (3) test-tubes of sterilized soil, transferred; (4) test-tubes of sterilized soil, not transferred. In preparing the soil tubes, sandy loam soil was sifted through a fine screen and added to test-tubes to within an inch of the top. To ensure a sufficient content of moisture about 2 cc. of water was added to each before they were plugged and sterilized. The slants and tubes were inoculated with freshly isolated single spore cultures of the Fusarium wild type to be studied and were transferred successively during a period of three months except for the set of soil tubes that was not transferred. There were three replicates of each combination of organism and cultural treatment. After the third transfer monosporous analyses were made of the contents of the tubes in the first replicate by adding sterilized distilled water to each and spreading the resulting suspension, diluted if necessary, over the surface of potato dextrose agar in Petri dishes from which single germinating spores were removed by the 'biscuit-cutter' technique. In the soil tubes, very few spores were produced and monohyphal isolates were

** This would appear to correspond to the "pionnotal" form of growth referred to by Wollenweber (42) as a stage with a maximum of conidia and a minimum of aerial mycelium. All the Fusaria studied here, when isolated from nature, produced on potato dextrose agar a form of growth with abundant aerial mycelium. But cultures of* F. udum *Butler isolated by the writer from material of* Cajanus *received through courtesy of Dr. B. B. Mundkur, New Delhi, India, showed a strongly "pionnotal" form of growth with abundant conidia and no aerial mycelium. It is evident that, by transferring, the initial growth form of the muskmelon* Fusarium *was changed to a type of growth resembling* F. udum, *and a danger of taxonomic confusion is apparent, if such transferring is not avoided.*

TABLE III

COMPARISON OF FOUR CULTURAL METHODS REGARDING THEIR EFFECT ON THE STABILITY OF 10 FUSARIA

Organism	Percentage of mutants isolated via 'biscuit cutter' technique											
	Potato dextrose agar slants			Soil agar slants			Sterilized soil in test tubes					
	Transferred			Transferred			Transferred			Not transferred		
	(1)*	(2)*	(3)*	(1)*	(2)*	(3)*	(1)*	(2)*	(3)*	(1)	(2)	(3)
Muskmelon *Fusarium*	100	100	100	0	100	0	0	0	—	0	0	0
Soybean *Fusarium*	0	100	100	4.5	0	0	0	0	0	0	0	0
Tomato *Fusarium*	85	100	100	27	18	96	0	0	0	0	0	0
Aster *Fusarium*	59	100	100	100	4.5	0	0	0	0	0	0	0
Navy bean *Fusarium*	72	100	100	0	0	0	0	0	0	0	0	0
Flax *Fusarium*	100	100	100	100	100	37	0	0	15	0	0	9
Cotton *Fusarium*	100	100	100	0	0	100	0	0	0	4	0	0
Soil Fusarium *A*	100	100	100	0	0	0	0	0	0	0	0	0
Soil Fusarium *B*	100	100	100	0	27	0	0	0	0	0	0	0
F. moniliforme	100	100	100	100	100	100	0	0	0	0	0	0

(1)* *Transferred three times.*
(2)* *Transferred four times.*
(3)* *Transferred five times.*

made by the same technique from hyphae that grew out of small grains of soil scattered sparsely over the surface of the agar. The same was done with the second replicate after the fourth transfer and after five transfers the third replicate was analysed. From each tube or slant a total of 22 'biscuit-cutter' isolations was attempted, a few of which usually failed to develop, and the resulting cultures, 2340 in all, were examined in search of mutants. The results thus obtained are summarized in Table IV.

In general the other nine Fusaria resembled the muskmelon *Fusarium* as regards degree of stability under the four types of cultural treatment. The wild types were not displaced as frequently on soil agar as on potato dextrose agar even though in the former medium many instances of loss of the wild type were noted. For the first time an instance of the loss of the wild type of the muskmelon *Fusarium* on soil agar slants was observed. In a previous publication (23) two experiments were described that included seven instances in which soil agar slants were inoculated with purified wild type cultures and transferred successively but in none of these was the wild type lost. Although the muskmelon *Fusarium* may be fairly stable on soil agar, this does not appear true for certain others, notably the tomato, aster, and flax Fusaria, and *F. moniliforme*. The soil tubes were definitely the most favourable medium for the maintenance of the wild types although even in these there was some indication of instability in the flax and cotton Fusaria, three tubes out of 12 yielding a low proportion of mutant cultures. The number of mutants thus obtained from soil tubes is too low for any conclusions to be

TABLE IV

COMPARISON, WITH RESPECT TO THE NUMBER OF MUTANT CULTURES PRODUCED, OF SLOWLY AND RAPIDLY GERMINATING SPORES* OBTAINED FROM PATCH MUTANTS OF NINE FUSARIA

Organism	Number of patch mutants involved	Slowly germinating spores		Rapidly germinating spores	
		Number of cultures obtained	Number that were mutants	Number of cultures obtained	Number that were mutants
Muskmelon *Fusarium*	5	61	35	62	43
Pea *Fusarium*	4	38	6	47	27
Soybean *Fusarium*	1	10	3	12	6
Tomato *Fusarium*	3	29	16	32	26
Aster *Fusarium*	1	12	0	11	7
Gladiolus *Fusarium*	1	8	0	13	3
Soil Fusarium *A*	1	9	2	5	4
Soil Fusarium *B*	2	20	5	18	15
F. moniliforme	1	10	0	13	5
Totals	19	197	67	213	136

* *Term includes both chlamydospores and conidia.*

drawn regarding the effect of transferring on the maintenance of the wild type under these conditions, but it would be reasonable to expect that successive transferring to fresh, moist tubes would give any mutants that did appear the opportunity to multiply, whereas this could hardly take place to advantage in a tube allowed to become dry and not transferred. Orton (26, 27) cultured *F. niveum* E.F.S. in flasks of sterilized soil and at monthly intervals removed samples of inoculum. In one experiment 41 out of 8220 differed from the parent culture.

The mutants obtained during this experiment were in general similar to those derived from patch mutants by monosporous isolations, lending support to the view that the degradation process is initiated by local mutations. The slant shown in Fig. 1 had been inoculated 21 days previously with a purified wild type culture of the muskmelon *Fusarium* in connection with the experiment just described. During this period two patch mutants (*A* and *B*) became evident and monosporous isolations from both of these yielded cultures of a light appressed mutant but similar isolations from a point midway between them yielded the wild type only. When, after three transfers, the spore suspension made from the contents of this tube was analysed, 20 out of 21 single spore cultures were of the light appressed type, which is strongly indicative of a connection between the patch mutants and the degradation process. A light semiraised mutant culture was also obtained, which is explicable on the basis of an additional mutation.

It is natural to wonder how a very small number of mutations, each arising presumably in a single cell of the mycelium, can eventually succeed in dominating the parent type or in displacing the latter completely. This problem

was discussed to some extent in a preceding publication (23) in which it was pointed out that the mycelium of certain mutant types of the muskmelon *Fusarium* was able to invade the areas occupied by the wild type along the line of contact, a phenomenon termed "cultural interaction." Monosporous isolations from this invaded zone yielded a much larger proportion of mutant cultures than of the wild type, indicating that the former was succeeding in dominating the parent in this region. The same process was assumed to account for the increase in size of the patch mutants. Transferring favoured the loss of the wild type perhaps because each transfer mixed the inoculum and as new growth resulted from this, the mutant would have additional opportunity to dominate adjacent wild type areas. In addition to this method of securing the ascendency over the parent type, evidence was obtained of a further method in the observation that, when monosporous isolations were made from patch mutants of the muskmelon *Fusarium*, the spores that germinated rapidly included a much higher proportion of mutants than those that germinated somewhat slowly. The conclusion seemed reasonable that when inoculum containing spores of both mutant and wild type is transferred, this rapidity of germination would give the former the advantage. During the present studies when monosporous isolations were made from patch mutants, spores that germinated relatively rapidly were kept separate from the slower ones in some instances and the number of mutant cultures in each was compared. The results, which are summarized in Table IV, indicate a correlation between the rapidity of germination of the spores and the number of mutants included, since from a total of 19 patch mutants 34% of the spores that germinated slowly produced mutant cultures whereas of those that germinated relatively rapidly 64% were mutants. Moreover, the table does not consider the case noted in *F. Scirpi* in which all of the spores obtained from a patch mutant germinated rapidly and gave rise to mutant cultures, as compared with those from an adjacent normal-appearing area, all of which germinated relatively slowly and produced the wild type.* Exceptions were provided by the flax and cabbage Fusaria in which the reverse seemed true but since only two patch mutants of the flax *Fusarium* and one of the cabbage *Fusarium* were studied in this regard, it is possible that more extensive data from these organisms would bring them into line with those in Table IV. The evidence provided by this table strongly suggests that rapidity of germination is a common occurrence in the spores of mutants derived from Fusarium wild types and accounts in part for the ease with which the latter are displaced in artificial culture. The existence of an additional means of accomplishing this end, as evidenced by the fact that

* *Rapidity of germination has not been found correlated with rapid growth rate in pure culture; no mutants were found that grew conspicuously faster than their respective wild types and frequently, as shown in the muskmelon* Fusarium *(23), they grew somewhat less rapidly. But a correlation has been noted between rapidity of germination and spore size, macroconidia usually germinating much more rapidly than microconidia, perhaps owing to the presence of a larger supply of readily available food material, which gives them an initial temporary advantage. Certain of the slow-growing mutants were found to invade adjacent cultures of the wild type producing conspicuous cultural interaction, which suggests that a slow growth rate would not hinder a mutant from dominating the wild type by this method.*

patch mutants increase in size owing to the invasion of the surrounding wild type by mutant material, has been pointed out above, and it seems logical to conclude that these two sets of observations account for the difficulty experienced in maintaining pure cultures of Fusarium wild types by the usual laboratory method.

The 10 Fusaria employed in the cultural degradation experiment yielded mutants that were of the same general types. Appressed mutants were the most common but semiraised and raised types were also found. In the potato dextrose agar slants the proportions were 34 appressed: 5 semiraised: 4 raised. The number of times that raised mutants were isolated from soil agar slants was higher, the proportions being 5 appressed: 1 semiraised: 7 raised. The indication is that the nature of the medium in some manner affects the type of mutant arising in it.

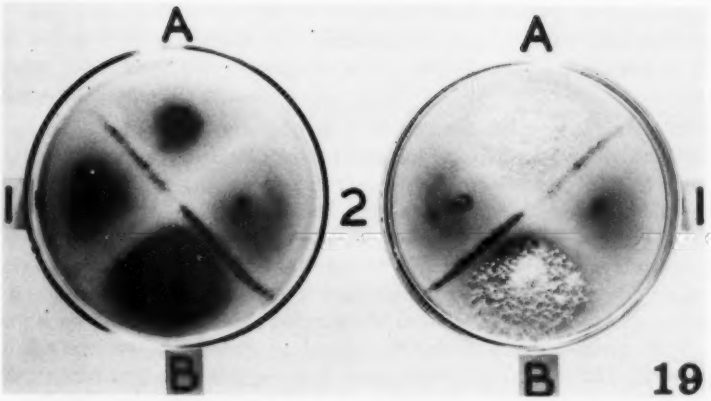
Macroconidia were not always abundant in the transferred slants but sometimes became very numerous, comprising up to 20% of the conidia present, and certain of the mutants obtained from the slants bore numerous and conspicuous macroconidia. These observations on spore production are of taxonomic interest. Wollenweber *et al.* (44) recommend transfers to obtain "normal" conidia for taxonomic purposes. Macroconidia, regarded by them as the "normal" type, have been obtained here by repeatedly transferring purified wild type cultures on potato dextrose agar. Since the Elegans wild types produced no macroconidia (or at least very few) on this medium and since monosporous isolations from the transferred slants consistently yielded mutant cultures many of which bore macroconidia, the conclusion seems evident that transferring favours macrospore production because it leads to the displacing of the wild types by such mutants. It would seem inadvisable to employ such macroconidia in taxonomic studies, a point that will be considered more fully in a subsequent publication.

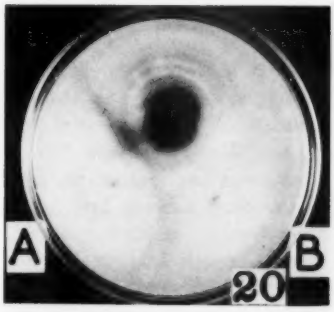
Cultural Interaction

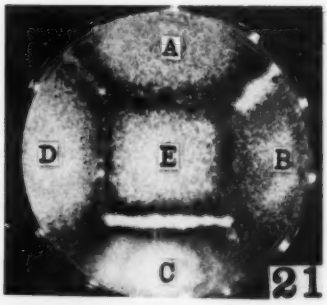
The phenomenon of cultural interaction has previously been described (23) and its possible value as a taxonomic criterion was indicated. These investigations have been carried further employing a number of Fusaria in an effort to assess the extent to which the phenomenon may be employed to identify Fusarium wild types.

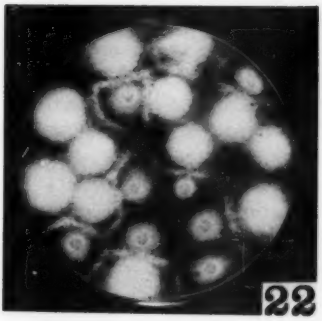
Definite interaction was observed in most of the Fusaria studied but the intensity of the effect varied greatly. The most striking examples of inter-

FIG. 19. *Cultural interaction between two Fusarium wild types and their derived mutants. A = cotton* Fusarium. *B = cowpea* Fusarium. *1 = light appressed mutant from A. 2 = light appressed mutant from B. The plate to the left is inverted.* FIG. 20. *Cultural interaction between two wild types and a mutant. A = Soil Fusarium A. B = Soil Fusarium B. Above is a dark appressed mutant derived from A. The plate is inverted.* FIG. 21. *Cultural interaction between isolates of the flax* Fusarium. FIG. 22. *Cultural interaction in the flax* Fusarium *between 5-day-old monosporous cultures of the wild type and a light semiraised mutant, all isolated from the patch mutant shown in Fig. 7.* FIG. 23. *Appearance two weeks later of the plate shown in Fig. 22.*


A
1
2
B
A
1
B
19


A
B
20


A
D
E
B
C
21


22

23

action were noted in the muskmelon, cabbage, flax, cotton, and cowpea Fusaria. Interaction was apparent but not usually conspicuous in the pea, soybean, and tomato Fusaria, in Soil Fusarium *A*, Soil Fusarium *B*, and in *F. moniliforme* and *F. Scirpi*. No evidence of the phenomenon was noted in the aster and navy bean Fusaria or in *Gibberella Saubinetii*. The intensity of the interaction in those Fusaria that displayed it varied greatly with different mutants, a point that has previously been pointed out in the case of the muskmelon *Fusarium* (23).

The possible value of interaction as a taxonomic criterion was tested by allowing mutants that gave pronounced interaction with their parent cultures to contact other wild types growing in the same Petri dishes. Fig. 19 shows an instance in which light appressed mutants of the cotton and cowpea Fusaria are matched with their respective wild types. The specificity displayed here is very marked, the interaction expressing itself as a dark band in the immersed mycelium between each wild type and its derived mutant but there is no suggestion of this between the cowpea wild type and the cotton mutant and vice versa. Similarly, in Fig. 20 a dark line is evident between Soil Fusarium *A* and a dark appressed mutant isolated from it but this is not evident between the mutant and Soil Fusarium *B*. Two additional experiments not illustrated here add further evidence for specificity. (1). In the flax *Fusarium*, cultural interaction was not noted between a light appressed mutant and the wild types of the muskmelon *Fusarium*, Soil Fusarium *A*, and Soil Fusarium *B* but it occurred consistently between this mutant and its parent wild type. (2). In the cabbage *Fusarium* a mutant that interacted strikingly with the parent failed to give any evidence of this effect when it was allowed to contact the muskmelon, pea, soybean, tomato, aster, navy bean, cotton, and cowpea Fusaria, Soil Fusarium *A* and Soil Fusarium *B*. These experiments taken in conjunction with those outlined in a preceding publication (23) suggest that the phenomenon is sufficiently specific to serve as an aid to taxonomy. It is particularly noteworthy that by this means it was possible to separate the two saprophytic Elegans Fusaria, since differential pathogenicity cannot be employed in such organisms to check a diagnosis.

Interaction is not confined to forms appearing in culture. In Fig. 21 are shown the results of an experiment in which a number of wild type strains of the flax *Fusarium** were allowed to contact each other. It will be noted that interaction was very marked between *A* and *B*, and *C* and *E*, and served to distinguish all the strains shown with the exception of *A* and *D*. In a separate experiment, *D* was found to interact with *B* much less strongly than did *A*. Thus all five strains shown can be separated by means of cultural interaction.

The expression of interaction between parent and mutant noted in the present studies may be divided into three main types: (1) that in which the predominant effect is the invasion of the wild type culture by the mutant,

* *Where reference is made elsewhere in this publication to the flax* Fusarium, *an isolate obtained in 1944 is indicated, and this differed in some respects from the strains shown in Fig. 21, which were isolated in 1945.*

exemplified by the muskmelon, pea, tomato, and cabbage Fusaria; (2) that in which a dark band appears between the two, exemplified by the cotton *Fusarium*, cowpea *Fusarium*, Soil Fusarium *A*, and on occasion by the muskmelon *Fusarium*; (3) that in which the wild type overgrows the mutant culture, the only instance of this type being the flax *Fusarium*. The latter type of interaction is illustrated in Figs. 22 and 23. Fig. 22 shows a plate of monosporous isolations obtained from the patch mutant in Fig. 7 and photographed five days after the isolations were made, at which time cultural interaction was just beginning to be evident. Fig. 23 shows the condition of the same plate two weeks later. During the interim the wild type cultures had overgrown more than half of the total area occupied by the mutants. Very little further progress in this regard was made and within 10 days after the photograph for Fig. 23 was taken, the ridge of aerial mycelium had collapsed.

Some workers might be inclined to interpret this marked mycelial growth at the point of contact as an example of increased mycelial vigour resulting from a heterocaryotic state. However, the results of certain investigations performed in this connection lend no support to the view. If a heterocaryotic state existed in this ridge of mycelium, then it would be expected that single hyphae isolated from it would, in a large proportion of cases, give rise to mixed cultures. Accordingly, mycelium was taken from the area in question and was macerated and teased out in order to make a suspension in sterilized distilled water. This was then spread over a plate of agar as in the technique for making monosporous isolations. Single hyphae about 5 to 10 cells long were then isolated via the 'biscuit-cutter' method and transferred to other plates. The cultures that grew from the hyphae were either wild type or mutant and the phenomenon was repeated where these contacted each other. In all, 48 single hyphae were isolated, 38 of which gave rise to wild type cultures and the remainder to the mutant. In no case was a mixed culture obtained and it thus seems unlikely that heterocaryosis can account for the phenomenon.

The explanation should, perhaps, be sought on ecological rather than on cytological grounds. The cultural environment encountered by the wild type of the flax *Fusarium* where it begins to overgrow the area occupied by the mutant would certainly differ from that in which it was growing up till then and it may well favour an intensified proliferation of aerial mycelium, intermingled with hyphae sent up from the mutant. A similar situation in more or less modified form is likely responsible for the other types of interaction described.

Discussion

(A) *The Nature of Variation in* Fusarium

Variation is a common phenomenon in the genus *Fusarium* and the variants described by other workers resemble those obtained here. The organisms seem to vary between a type with abundant aerial growth and a type in which

all the mycelial growth is immersed in the nutrient substrate. Differences in colour and in the amount and type of sporulation are frequent.

Three main theories have been advanced to account for these variations.

The cyclogenic variation theory as proposed by Leonian (17, 18) represents an effort to account for the appearance of different cultural forms by assuming them to be merely phases in the orbit of variation of a species. As evidence for this he points out the manner in which Fusarium isolates were found to fluctuate back and forth during transfers. Since no effort was made to purify the inoculum employed in the transfers, it is not surprising that such fluctuations were observed, and rather than establishing the cyclogenic hypothesis the observations may merely indicate that the inoculum was often a mixture of two or more strains, the proportions of which varied with successive transfers.

Similar views have been expressed by Brierley and Goddard. In 1931 Brierley (6) objected to the term 'mutation' as applied to fungi since he considered that at that time the knowledge of the genetics and cytology of fungi was insufficient to justify it. In his opinion most of the variation phenomena were merely selective eliminations or combinative, dissociative, segregative, or cyclogenic changes originating in one form or another of sexual or somatic fusion. He considered hyphal fusions to be more important than we realize, but did not explain how these could induce changes in the progeny of a single, haploid, uninucleate spore. Goddard (13) concluded that there was a definite cycle of growth phases through which the fungus passed. She was able to cause five out of eight variants of *Gibberella Saubinetii* to revert to the parent type by transferring them from potato dextrose agar to Leonian's medium. However, the variants were not purified before this transfer and it seems not at all impossible that the inoculum included some of the parent type, which was able to predominate in the new environment.

If 'cyclogenic variation' were responsible for the appearance of variants in Fusarium cultures, then reversion to the parent type should be a common phenomenon, but it must be emphasized in this connection that in the writer's experience once a variant was purified by the monosporous (or monohyphal) method, reversion to the parent type never followed. Wellman and Blaisdell (40) and Wellman (38) obtained some degree of reversion with the tomato wilt *Fusarium*, but Brown (8) found no evidence of reversion in the *Fusarium* with which he worked, and Armstrong *et al.* (3) did not observe reversion in *Fusarium vasinfectum* Atk. It therefore seems advisable to look elsewhere for the cause of variability in *Fusarium*.

Variability in many of the Fungi Imperfecti can be explained, according to Hansen (14), on the basis of what he terms the "dual phenomenon." This assumes variation to be the result of a segregating out of genetically different nuclei in a multinucleate mycelium. In a preceding publication Hansen and Smith (15) reported variations in *Botrytis cinerea* Pers. that they attributed to nuclear segregation. If we assume the "dual phenomenon" operates in a

multinucleate form like *Botrytis*, the theory meets difficulties when efforts are made to apply it to *Fusarium*, since a prerequisite of the theory is that the cells of the organism concerned must be multinucleate. This does not seem to be the case in *Fusarium*. It was shown in the muskmelon *Fusarium* (23) that most of the mycelial cells and the microspores were uninucleate. Dickinson (10) studied two species of *Fusarium* and found that each cell in both the mycelium and the conidia contained one nucleus. It is thus impossible to account for the production of variants among the progeny of a single spore by the segregating out of mixed nuclei, and heterocaryosis can hardly be responsible for the sort of variation found in *Fusarium*. Yet Hansen (14), referring to the work of Leonian (17), makes the statement that the cultural variants shown in certain of Leonian's figures "are all most excellent demonstrations of the dual phenomenon." The observations of the present studies suggest a simpler explanation for the sectoring observed: that the inoculum that produced these cultures was a mixture of two types, one or both of which had originated through mutation in culture.

In attempting to apply the "dual phenomenon" to many of the Fungi Imperfecti, Hansen (14) lists over 30 genera, over half of which when analysed by the single spore method were found to be "dual," i.e. composed of two culturally distinct individuals. An intermediate type was also found, which broke down into the two "homotypes." To support this cytologically he states that the spores of *Botrytis* are multinucleate, those of *Phoma terrestris* Han. binucleate, and those of *Verticillium albo-atrum* R. & B. mainly uninucleate. Again, the spores of *Ascochyta Pisi* Lib. are stated to contain seldom more than seven nuclei. But work by Ludwig (21) has indicated that the sporophores of *Ascochyta Pisi* Lib. contain only one nucleus. Dickinson (10) has shown in *Fusarium* and *Helminthosporium* that all the nuclei in a multinucleate or multicellular spore arise from a single parent nucleus, and this point has been confirmed by the writer with *Fusarium*. Hence in the latter three organisms the carryover of a mixed nuclear condition from a heterocaryotic mycelium would be unlikely, although Hansen concludes that this is a common occurrence in *Ascochyta Pisi* Lib. as well as in many other fungi with multinucleate spores.

It would appear that Hansen has failed to give full consideration to the possibility that the variations observed are susceptible to explanation on the basis of mutations in culture, which may be a result of working first with a form like *Botrytis* rather than with a uninucleate form. It would seem more reasonable to study first forms possessing essentially uninucleate cells and thus obtain a basis for reasoning that may be applied to the more complex situation that could exist in multinucleate forms, rather than vice versa.

The weight of evidence accumulated to date definitely favours the view that mutations are responsible for cultural variation of the sort observed in *Fusarium*. It is difficult to conceive how variants may arise among the progeny of a single, haploid, uninucleate spore unless by mutation. Ullstrup (36) and Eide (12) obtained variants in cultures that arose from single asco-

spores of *Gibberella Saubinetii*. Further evidence is provided by the genetic behaviour shown by certain cultural variants. The work of Dimock (11) and of Hansen and Snyder (16) on *Hypomyces* has shown that variants may be inherited as unit characters. Stakman *et al.* (35) have shown mutability and constancy in haploid lines of *Ustilago Zeae* (Beckm.) Ung. to be due to genetic factors. Mention should also be made of the work of Whitehouse (41) and of Lindegren (19) who have demonstrated crossing-over and mapped chromosomes by employing cultural variants of *Neurospora*. Likewise, Shay and Keitt (29) have shown certain mutants that appeared in cultures of *Venturia inaequalis* (Cke.) Wint. to be inherited as unit characters. Beadle and Tatum (4) describe work on *Neurospora* in which by irradiation of spores with X-rays, ultraviolet radiation, or neutrons, mutants differing in their nutritional requirements were obtained. Genetic studies showed these to differ by single genes from the wild type strains from which they were obtained.

(B) *Variation in Nature*

Most of the Fusaria studied by the writer to date, when first isolated from plants infected in naturally infested soil, were of a type that produced abundant aerial mycelium on potato dextrose agar. When these were grown for a time on this medium and perhaps transferred several times it was commonly noted that the original mycelial type was displaced by mutants that formed less aerial mycelium and, occasionally, more and larger spores. The fact that the original isolates were not of the appressed type is sound evidence that this process does not occur in the soil. Two reasons may be advanced to account for this. First, the organisms are genetically more stable in the soil than in artificial culture. Evidence to support this is provided by the experiments on cultural degradation, which showed that far fewer mutations appeared in a nutrient medium approximating that of the soil environment, i.e., in tubes of sterilized soil. Second, if variants of the sort that commonly appear in culture do arise in the field they may be less able to survive the competition of other organisms and hence would tend to be crowded out. In this connection it was shown in the case of the muskmelon *Fusarium* (23) that certain mutants that were almost as virulent as the wild type in sterilized soil were definitely less virulent in unsterilized soil and in the field. When such mutants were introduced into the field along with the wild type it was found that after a year they were negligible as a disease factor but the wild type still caused wilt.

That the original isolates of many Fusaria are of a type with abundant aerial mycelium is also evident from the observations of other workers. Weindling (37) made a careful study of 13 monosporous isolates of *Fusarium vasinfectum* Atk. and found that all the original isolates from diseased plants were alike and had abundant aerial mycelium. They were all highly pathogenic and tended to be crowded out by less virulent, more appressed variants. All the isolates of *Gibberella Saubinetii* that Ullstrup (36) obtained from perithecia on diseased plants were similar and variants appeared when these

were subcultured. Brown (8) noted that most of his original isolates of *Fusarium fructigenum* Fr. were of the mycelial type and produced small spores. Appel and Wollenweber (2) in describing the basis for the taxonomy of the genus pointed out that many Fusaria as isolated from nature developed aerial mycelium with small, low-septate spores. Wollenweber *et al.* (44), in discussing the classification of Fusaria, advise various cultural treatments to obtain a type of growth producing "normal macroconidia" in the case of those species the isolates of which persistently produce only microconidia. The evidence seems conclusive therefore that many, if not most, Fusaria occur in nature as types that may be altered by a period of growth in culture.

It is not implied that no variation takes place in the field but this must be relatively infrequent and the new forms that may thus become established tend to resemble the parent, the range of variability evidently being far narrower than that observed in artificial culture. For example, while all the initial isolates of the muskmelon *Fusarium* were identical, this did not hold true for the flax *Fusarium*. Over 200 single-spore isolates were obtained in 1945 from surface sporulation on 25 flax plants, and while all the isolates from each plant were usually identical, some cultural differences were noted between those from different plants, at least five types being distinguishable (Fig. 21). Borlaug (5) found a large number of cultural races of *F. Lini* attacking flax in Minnesota. Nelson *et al.* (25) described two forms of *Fusarium Apii* Nelson and Sherbakoff as causing celery yellows in Michigan. Both forms had abundant aerial mycelium and could be distinguished with difficulty in culture. Symptoms characteristic of each were recognizable in the field and often only one form was present in a region where the disease had recently been introduced. Thus it is evident that more than one wild type (or form) may occur in the field. The additional possibility that a number of pathogenic forms may be included should also be considered. Broadfoot (7) found nine forms of *F. Lini* among isolates from various localities. These were very difficult to distinguish in culture but they could be separated by their relative virulence on four flax varieties. Likewise, Borlaug (5) found a number of pathogenic races among his isolates of *F. Lini*, and showed them to be of importance under field conditions. Alexander and Tucker (1) isolated a race of the tomato wilt *Fusarium* in Ohio to which varieties hitherto resistant were found to be completely susceptible. The bearing of the wild type concept on the problem of breeding for resistance has been discussed by the writer in Part II of *Studies on the Fusarium of Muskmelon wilt* (24).

Views differing from those outlined above have been expressed by certain other authors. Sleeth (31) observed a wide range of variation among isolates of the watermelon wilt *Fusarium* from various parts of the United States and assumed that these occurred naturally. A critical examination of the cultural methods he employed casts doubt upon this assertion. For example, some of the isolates were cultured for several years and no mention is made of any effort to maintain them in a pure state. In view of the observations

described here with certain other wilt Fusaria, it would be remarkable indeed if during this time they had not changed. Likewise, Wellman and Blaisdell (39) compared 30 isolates of the tomato wilt *Fusarium*, three of which had been in culture prior to 1924, and most of the remainder for at least two years. As would be expected, marked differences both in cultural appearance and in virulence were found among the cultures, but the assumption that these existed as such in the field seems unwarranted. Snyder (32) found similar wide variation among isolates of the pea wilt *Fusarium* and stated the original isolates to show as wide a range of variability as that derived in culture from the progeny of a single spore. Here again the failure to compare the cultures immediately after isolation or to adopt measures to prevent the original cultures from being displaced by mutants vitiates the conclusion that this sort of variation occurs in the field. Both Wellman and Blaisdell, and Snyder, noted differences in virulence among their cultures but no host specialization was found. Armstrong *et al.* (3) found marked differences in pathogenicity and cultural characteristics between isolates of *Fusarium vasinfectum* Atk. from different localities but these authors recognize the fact that the weakly pathogenic, appressed cultures may have originated during the lengthy period in culture. Fresh isolations from diseased plants in the field were characterized by abundant aerial mycelium.

It cannot be overemphasized that in studies concerning the variability of Fusaria in nature, cultural comparisons should be made as soon as possible after isolation unless particular care is taken to maintain the purity of the originals. If differences in cultural appearance or host specialization (or both) are noted among the original isolates, these should be regarded as separate wild types of the organism concerned. Mutant strains that may arise when the wild types are cultured on most laboratory media need not be considered seriously either in pathogenicity studies or in the taxonomy of *Fusarium*.

(C) *The Relation of Variation to Taxonomy*

The importance of the preceding conclusion to Fusarium taxonomy is obvious. If a consistent morphological treatment of this difficult group of fungi is to be achieved, care must be taken that the features studied and compared are those of the wild types.

In this connection the problem of maintaining stock cultures is of prime importance and it is felt that the experiments on cultural degradation described in the present studies and in the preceding work on the muskmelon *Fusarium* (23) will provide an answer. The observation was recorded that the wild types were readily maintained in tubes of sterilized soil with little danger of being displaced by mutants, which so frequently happened in potato dextrose agar and to a lesser extent in soil agar. These soil tubes were found to provide a very convenient source of inoculum, wild type cultures being obtainable at will by transferring a few grains of dry soil to the agar medium.

It will not be out of place to describe at this point an experiment involving certain soil tube cultures of the muskmelon wilt *Fusarium*. In August, 1942, eight tubes, which included four soil types, were inoculated with a recently isolated culture of the organism. For a month they were kept in a moist atmosphere and were then allowed to become dry. In August, 1945, transfers of soil grains to potato dextrose agar were made and the organism was found to be still viable in all tubes. Single spore cultures were made from two of the cultures thus obtained and were compared in the same Petri dish with single spore cultures from two isolates recently obtained from diseased muskmelons. All four sets of single spore cultures appeared identical. Thus the cultural expression of the wild type had remained unchanged in dry soil for three years, and it was left to determine whether its pathogenicity had been affected. To investigate this, a greenhouse infection experiment was performed, which included four single-spore cultures, one from each of the four sets mentioned. The technique of the experiment followed that employed in the previous studies on the muskmelon wilt *Fusarium* (23). Fifty seeds (variety Hoodoo) were planted per flat and emergence in the two check flats was very high (98%). Fifteen days after the melon seeds were planted the percentage of mortality (including both pre- and post-emergence killing) caused by the two cultures from the soil tubes was 88 and 90%, respectively, and that caused by the two 1945 isolates 82 and 84%. Twenty days after planting these percentages were 100, 98 and 98, 100, respectively. It may therefore be concluded that the wild type underwent no change in cultural form and suffered no loss of virulence during three years in dry soil.

The other Fusaria studied here have not been investigated as thoroughly as the muskmelon *Fusarium*, but it may be stated that the wild types of the soybean, tomato, aster, navy bean, flax, cotton, and cowpea Fusaria were still viable in similar tubes after 19 months. *Fusarium moniliforme* was found to be still viable after 15 months, and the two soil Fusaria after 13 months.

To replace the normal laboratory technique of maintaining stock cultures of Fusaria by serial transfers on media high in nutrient, the following procedure is suggested. Inoculate tubes of moist sterilized soil with recently obtained mass isolates or single spore cultures and allow contents to become dry. Transfer once a year to a fresh tube of soil. As a safeguard the purity may be checked at each transfer. Such tubes should provide a reliable source of inoculum for studies on pathogenic behaviour and on taxonomy.

With regard to the problem of exsiccatae it does not seem unreasonable that soil cultures could serve as type specimens for herbaria. Wollenweber *et al.* (44) state that pure cultures of each of the Fusaria studied at the Fusarium Conference would be placed in the Bureau of Plant Industry, U. S. D. A. and would have substantially the status of type specimens. It is difficult to conceive of what taxonomic value such cultures could be, particularly since the "normal culture" technique of the authors probably resulted in the loss of the wild types even before the cultures were placed in the Bureau. Rather, it reaffirms the necessity of developing some convenient method for

maintaining the wild types in the pure state, if a satisfactory degree of uniformity is to be introduced into the taxonomy of the genus.

Acknowledgments

The writer wishes to express his sincere thanks to Dr. L. W. Koch, Officer-in-Charge of the Dominion Laboratory of Plant Pathology, Harrow, Ont., for rendering available the facilities for this work and for his constant interest during the course of the investigations. The contributions of diseased specimens and cultures have greatly increased the scope and value of these studies, and the writer is very grateful to those who assisted in this regard. Thanks are due to Prof. D. L. Bailey of the Department of Botany, University of Toronto, and to Drs. L. W. Koch and A. A. Hildebrand of the Dominion Laboratory of Plant Pathology, Harrow, for valuable suggestions both during these studies and following the preparation of the manuscript in its initial form.

References

1. ALEXANDER, L. J. and TUCKER, C. M. Physiologic specialization in the tomato wilt fungus *Fusarium oxysporum* f. *lycopersici*. J. Agr. Research, 70 : 303-313. 1945.
2. APPEL, O. and WOLLENWEBER, H. W. Grundlagen einer Monographie der Gattung *Fusarium* (Link). Arb. Kaiserl. biol. Anstalt Land-Forstw. 8 : 1-207. 1910.
3. ARMSTRONG, G. M., MACLACHLAN, J. D., and WEINDLING, R. Variation in pathogenicity and cultural characteristics of the cotton-wilt organism, *Fusarium vasinfectum*. Phytopathology, 30 : 515-520. 1940.
4. BEADLE, G. W. and TATUM, E. L. *Neurospora*. II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Botany, 32: 678-686. 1945.
5. BORLAUG, N. E. Variation and variability of *Fusarium lini*. Minn. Agr. Expt. Sta. Tech. Bull. 168. 1945.
6. BRIERLEY, W. B. Biological races in fungi and their significance in evolution. Ann. Applied Biol. 18 : 420-434. 1931.
7. BROADFOOT, W. C. Studies on the parasitism of *Fusarium lini* Bolley. Phytopathology, 16 : 951-978. 1926.
8. BROWN, W. Studies in the genus *Fusarium*. VI. General description of strains, together with a discussion of the principles at present adopted in the classification of *Fusarium*. Ann. Botany, 42 : 285-304. 1928.
9. BROWN, W. and HORNE, A. S. Studies in the genus *Fusarium*. III. An analysis of factors which determine certain microscopic features of *Fusarium* strains. Ann. Botany, 40 : 203-243. 1926.
10. DICKINSON, S. The nature of saltation in *Fusarium* and *Helminthosporium*. Minn. Agr. Expt. Sta. Tech. Bull. 88. 1932.
11. DIMOCK, A. W. Hybridization experiments with natural variants of *Hypomyces ipomoeae*. Bull. Torrey Bot. Club, 64 : 499-507. 1937.
12. EIDE, C. J. The pathogenicity and genetics of *Gibberella saubinetii* (Mont.) Sacc. Minn. Agr. Expt. Sta. Tech. Bull. 106. 1935.
13. GODDARD, M. Studies on variation in *Gibberella saubinetii* (Mont.) Sacc. Ann. Missouri Botan. Garden, 26 : 99-164. 1939.
14. HANSEN, H. N. The dual phenomenon in imperfect fungi. Mycologia, 30 : 442-455. 1938.
15. HANSEN, H. N. and SMITH, R. E. The mechanism of variation in imperfect fungi: *Botrytis cinerea*. Phytopathology, 22 : 953-964. 1932.
16. HANSEN, H. N. and SNYDER, W. C. The dual phenomenon and sex in *Hypomyces solani* f. *cucurbitae*. Am. J. Botany, 30 : 419-422. 1943.
17. LEONIAN, L. H. Studies on the variability and dissociations in the genus *Fusarium*. Phytopathology, 19 : 753-868. 1929.

18. LEONIAN, L. H. The pathogenicity and variability of *Fusarium moniliforme* from corn. West Virginia Agr. Expt. Sta. Bull. 248. 1932.

19. LINDEGREN, C. C. A six-point map of the sex-chromosome of *Neurospora crassa*. J. Genetics, 32 : 243-256. 1936.

20. LUCAS, G. B. Further studies on the deterioration of the red rot fungus in culture. Proc. Louisiana Acad. Sci. 7 : 35. 1942.

21. LUDWIG, O. Untersuchungen an *Ascochyta Pisi* Lib. Beitr. Biol. Pflanzen, 16 : 464-510. 1928.

22. MCLAUCHLIN, A. M. A Fusarium disease of *Cereus schottii*. Phytopathology, 24 : 495-506. 1934.

23. MILLER, J. J. Studies on the *Fusarium* of muskmelon wilt. I. Pathogenic and cultural studies with particular reference to the cause and nature of variation in the causal organism. Can. J. Research, C, 23 : 16-43. 1945.

24. MILLER, J. J. Studies on the *Fusarium* of muskmelon wilt. II. Infection studies concerning the host range of the organism and the effect of environment on disease incidence. Can. J. Research, C, 23 : 166-187. 1945.

25. NELSON, R., Coons, G. H., and COCHRAN, L. C. The Fusarium yellows disease of celery. Mich. Agr. Expt. Sta. Tech. Bull. 155. 1937.

26. ORTON, C. R. Dissociation of *Fusarium niveum* in soil. Phytopathology, 25 : 30. 1935.

27. ORTON, C. R. Dissociation of *Fusarium* in soil. Bull. Torrey Botan. Club, 62 : 413-420. 1935.

28. RIKER, A. J. and RIKER, R. S. Introduction to research on plant diseases. John S. Swift Company, Inc., St. Louis, Mo. 1936.

29. SHAY, J. R. and KEITT, G. W. The inheritance of certain mutant characters in *Venturia inaequalis*. J. Agr. Research, 70 : 31-41. 1945.

30. SHERBAKOFF, C. D. Fusaria of potatoes. N.Y. Cornell Agr. Expt. Sta. Memoirs, 6 : 89-270. 1915.

31. SLEETH, B. *Fusarium niveum*, the cause of watermelon wilt. West Virginia Agr. Expt. Sta. Bull. 257. 1934.

32. SNYDER, W. C. Variability in the pea wilt organism, *Fusarium orthoceras* var. *Pisi*. J. Agr. Research, 47 : 65-88. 1933.

33. SNYDER, W. C. Notes on Fusaria of the Section Martiella. Zentr. Bakt. 91 : 163-184. 1934.

34. SNYDER, W. C. and HANSEN, H. N. The species concept in *Fusarium*. Am. J. Botany, 27 : 64-67. 1940.

35. STAKMAN, E. C., KERNKAMP, M. F., KING, T. H., and MARTIN, W. J. Genetic factors for mutability and mutant characters in *Ustilago zeae*. Am. J. Botany, 30 : 37-48. 1943.

36. ULLSTRUP, A. J. Studies on the variability of pathogenicity and cultural characters of *Gibberella Saubinetii*. J. Agr. Research, 51 : 145-162. 1935.

37. WEINDLING, R. Variability of *Fusarium vasinfectum* in culture. Phytopathology, 29 : 755. 1939.

38. WELLMAN, F. L. Increase of pathogenicity in tomato-wilt *Fusarium*. Phytopathology, 33 : 175-193. 1943.

39. WELLMAN, F. L. and BLAISDELL, D. J. Differences in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U.S. Dept. Agr. Tech. Bull. 705. 1940.

40. WELLMAN, F. L. and BLAISDELL, D. J. Pathogenic and cultural variation among single-spore isolates from strains of the tomato wilt *Fusarium*. Phytopathology, 31 : 103-120. 1941.

41. WHITEHOUSE, H. L. K. Crossing-over in *Neurospora*. New Phytologist, 41 : 23-62. 1942.

42. WOLLENWEBER, H. W. Studies on the Fusarium problem. Phytopathology, 3 : 24-50. 1913.

43. WOLLENWEBER, H. W. and REINKING, O. A. Die Fusarien. Paul Parey, Berlin. 1935.

44. WOLLENWEBER, H. W., SHERBAKOFF, C. D., REINKING, O. A., JOHANN, H., and BAILEY, A. A. Fundamentals for taxonomic studies of *Fusarium*. J. Agr. Research, 30 : 833-843. 1925.

CULTURAL AND TAXONOMIC STUDIES ON CERTAIN FUSARIA

II. THE TAXONOMIC PROBLEM IN *FUSARIUM* WITH PARTICULAR REFERENCE TO SECTION ELEGANS[1]

By J. J. Miller[2]

Abstract

The conclusions that the 'normal culture' stage described by certain specialists in Fusarium taxonomy represents the dominance of the original or wild type by a mutant, and that the 'sporodochia' that appear in laboratory cultures of *Fusarium* are actually patch mutants are shown to have an important bearing on the taxonomy of the genus. The indication is that the species descriptions of many Fusaria are based to a large extent on cultural variants of the types found in nature. It is emphasized that in order to avoid confusion, care should be taken to base species descriptions on the characters of the wild types. Further, while morphological separation of wild types may be found extremely difficult within certain groups of Fusaria owing to the close similarity of the components, the effort should not be abandoned because of the wide range of variation displayed by these organisms in artificial culture. Species should be merged only when morphological separation is extremely difficult or where variability *in nature* makes consistent diagnosis uncertain.

Introduction

Basic to a useful systematic study of any group of organisms is an understanding of the degree of variation that may be expected among them in consequence of environmental or genetic differences. In *Fusarium* it would appear that these two sources of variation are rather closely associated since, in addition to the fact that the appearance of the mycelium and the character of the sporulation of a given pure culture may be markedly affected by different types of cultural environment, certain cultural media favour the appearance of mutations that may soon dominate the original. In preceding publications of the writer (3, 4, 5) it was concluded that the taxonomy of the genus should be based on the types found to occur in nature, since otherwise a great deal of confusion might easily ensue. The phenomenon of 'cultural degradation' has, therefore, an important bearing on the problem of Fusarium taxonomy in two respects: (1) past systematic work on the genus should be critically examined from this point of view, and (2) future descriptions of species of *Fusarium* should be prepared in full cognizance of the confusion that may result through mutation in pure culture.

The Classification of *Fusarium*

Two major systems of Fusarium taxonomy are in existence; that embodied by Wollenweber and Reinking in their classic monograph *Die Fusarien* (14),

[1] *Manuscript received May 9, 1946.*
Contribution No. 871, Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, Canada.
[2] *Agricultural Assistant, Dominion Laboratory of Plant Pathology, Harrow, Ont.*

and the subsequent revision of the genus by Snyder and Hansen (10, 11, 12) in which the 65 species listed by Wollenweber and Reinking were reduced to 10.

The former of these is based primarily on the work of Appel and Wollenweber (1), Sherbakoff (9), and Wollenweber *et al.* (15). These authors take the view that Fusaria when first isolated from nature may be in a state unsuited to taxonomic work, the so-called "Ankultur", because the conidia are irregular in shape and septation. By growing them in culture a further stage, termed "Normkultur", is obtained, which is characterized by the production of so-called "normal" conidia. As Sherbakoff (9) states: "A culture is considered to be a normal one (*Normkultur*) when all forms typical of the fungus—and especially the most important form, the macroconidia,—are abundant, comparatively uniform in size and shape, smooth in outline, and so forth." He considers a culture to be in the state of undevelopment or "Ankultur" when mycelial growth predominates accompanied by poor production of spores: "This state of culture may sometimes exist when a fungus is transferred from a mycelial growth, especially when it is taken directly from the host tissues." Again, Wollenweber *et al.* (15) take the view that generally the "normal condition of Fusaria from the standpoint of determination" is not present in nature but must be produced by growing the fungi in pure culture. Certain Fusaria, including *F. Scirpi*, are considered to be identifiable as they occur in nature, but "Other Fusaria, which appear primarily in a microconidial stage under natural conditions and produce only a few sickle-shaped spores, are more difficult to identify. For these the so-called normal growth must, at first, be produced." The methods proposed to effect this involve culturing the fungi over a period of time, species that persistently produce only microconidia often requiring special treatment such as repeated transfer of occasionally found macroconidia, which may finally lead to the abundant production of "normal macroconidia".

When these observations are viewed in the light of the present series, the conclusion will at once be apparent that these authors are actually dealing with genetic variants appearing in originally pure cultures. The statement of Wollenweber *et al.* (15) that "the type of inoculum influences the resultant growth" is clearly indicative of genetic differences between adjacent regions in the cultures studied.

The so-called 'sporodochia' appearing in cultures of *Fusarium* are also regarded as a useful source of macroconidia for taxonomic purposes, but evidence presented in the preceding paper of this series (5) pointed to the conclusion that these are actually 'patch mutants' that result from the invasion of the surrounding wild type by mutants. Indeed, the grouping of *Fusarium* with the Tuberculariaceae of the Fungi Imperfecti may be called into question on this basis, since it would obviously be unreasonable to group *Fusarium* with fungi that form true sporodochia merely because of a superficial resemblance of the patch mutants to sporodochia. However, some Fusaria, notably the flax wilt organism, form true sporodochia on the stems of the host, but in the muskmelon *Fusarium*, although surface sporulation on

the host may be abundant, it is commonly expressed as a fairly uniform field without definite boundaries. Possibly the explanation lies in the fact that the hard outer layers of the flax stem so hinder the passage of the hyphae of the pathogen that they are able to emerge only at weak points, the resultant localization imparting a sporodochial appearance to the tufts of sporulation. On the other hand, both organisms show the same type of growth and sporulation in culture, the conidia being borne uniformly over the mycelium with no evidence of localization. If then, we are to include these two organisms in the same genus, or even family, it would seem necessary to base the grouping on the form of growth expressed in culture, in which event the genus would properly belong in the Moniliaceae.

The importance to taxonomy within the genus of the preceding conclusions that 'normal' cultures represent the dominance of the wild type by macrospore-bearing mutants and that the 'sporodochia' are actually 'patch mutants' cannot be underestimated. Brown (2) has suggested that the transfer method of obtaining macroconidia results in the elimination of the parent by a saltant. He also points out that since the appressed forms with long conidia tend to be feebly, if at all, parasitic, it seems unreasonable to classify pathogenic Fusaria on this basis. If the type of macroconidium present at 'normal culture' were always the same, an objection of this nature would be mainly academic. There seems no reason, however, for expecting all mutants to produce the same type of spore, nor is this found to be the case. Not only have the macroconidia produced by different mutants of a given Elegans *Fusarium* been observed to differ in length, width, and degree of curvature, but the same mutant may produce a different type of macroconidium depending on whether it is in the pure state or mixed with the wild type in a patch mutant. The conclusion therefore seems valid that the search for macroconidia could lead to confusion in the species descriptions since there is no assurance that every investigator will obtain the same type of macroconidium, or even that the same investigator might not obtain different types in separate isolates of the same organism.

Additional evidence in support of this conclusion is provided by certain statements in the Fusarium literature. Wollenweber *et al.* (15) indicate that they are aware of the possibility of confusion and comment: "It should be stated also that even conidia normal in appearance often differ greatly in their size and shape according to where they are produced, whether on mycelium, over a wet surface, or in a definite sporodochium. In a typical pionnotes long conidia may be produced, and in sporodochia they may be considerably shorter". Similarly Reinking and Wollenweber (8) state: "Sometimes, spores of particular species (*Fusarium cubense*) may have slenderer and longer spores in pionnotal masses than when produced from the mycelium or in older sporodochial masses." And also: "The measurements of conidia on different media, especially when the spores were taken from different sources, such as mycelium, pionnotes, or sporodochia, are significant in that they point out the possible differences that may arise within the same

species when normal spores are produced under various conditions." Thus, even if it were conceded that the 'normal stage' should be employed in Fusarium taxonomy, the problem of deciding which type of macroconidium should be regarded as 'normal' would still have to be faced.

In this respect the views expressed in the literature are for the most part disappointingly vague. Sherbakoff (9) recommends growing the fungi "for a long period of time, under different cultural conditions and from different kinds of material for inocula. Then, after observation of all the stages, it will be possible to pick out easily the typical and normal one and base judgment mainly on that." Wollenweber (13) states that the normal stage can be recognized because it "has the highest constancy in average size, curvature, septation, colour, and is further characterized by longevity." Wollenweber *et al.* (15) do not appear as confident that the problem can be easily solved but advise taking an average: "it is necessary to pay particular attention to the average size of different types as grown upon different media and under different conditions." The impractical nature of this advice is obvious since the determination of a single Fusarium isolate may become a research problem in itself.

The attitude basic to the systematic treatment of the genus in "Die Fusarien" may be summed up in the words of Wollenweber *et al.* (15): "The ordinary procedure in determining Fusaria is to illustrate and measure normal spores and a few exceptions that show the variability and changes with increasing age." In view of the foregoing discussion it is permissible to question whether the descriptions given in "Die Fusarien" can be of much value in the identification of those Fusaria that do not produce macroconidia as isolated from nature. The so-called "Ankultur", which is characterized by abundant mycelial production and small conidia, would correspond to what is here termed the "wild type", and hence it appears that the 'normal macroconidium' technique has led to the exclusion of the wild types of many Fusaria from the species descriptions.

Snyder and Hansen (10, 11, 12), realizing that isolates of *Fusarium* may show a wide range of variation in culture, have proposed a simplification of the Wollenweber–Reinking classification. They found that the variation in the progeny of single spore cultures of certain Elegans Fusaria was so great that they could be fitted into more than one species or section of the genus, and a morphological separation of these forms was concluded to be impractical. They therefore converted the section Elegans into a single species: *Fusarium oxysporum*, containing a large number of biologic forms that may be distinguished by pathogenicity tests. Subsequently the remainder of the genus was similarly revised.

The conclusions drawn by the writer in the present and preceding studies (3, 4, 5) do not support the thesis of Snyder and Hansen in all respects. It is agreed that the cultural variants of Elegans Fusaria may overlap, but the possibility that the original or wild types may be morphologically separable should be given serious consideration. If this is found to be the case, the

Snyder and Hansen classification will certainly be unsatisfying to the taxonomist. In this connection it must be pointed out that the observations of the present series provide strong support for the wild type concept. The initial isolates of all 13 Elegans Fusaria studied were of the 'raised' type and between them differences were apparent that, though often small, were consistently maintained provided care was taken to prevent the originals from being displaced by mutants. With the muskmelon *Fusarium* the writer (3) has shown that not only were the cultural variants of this organism consistently less virulent than the wild type, but when such mutants were actually introduced into the field along with the wild type, after a year they were found to be negligible as a disease factor but the wild type still caused wilt. The fact that the organisms were found to be more stable in sterilized soil than on a commonly-employed laboratory medium (potato dextrose agar) is evidence for stability in nature. The Ankultur–Normkultur technique of the earlier workers is further evidence for the validity of the wild type concept. The fact that they found it necessary to transfer or otherwise manipulate the initial isolates of many Fusaria before a 'stage' producing 'normal macroconidia' could be obtained indicates that the organisms exist in nature as forms that may be altered by a period of growth in culture.

The crowding-out of the original types in culture by mutants is obviously a very widespread phenomenon in the genus and it may be questioned whether the different morphological types that thus appear are of any great taxonomic significance. In the taxonomy of *Drosophila*, for instance, the variants obtained in the laboratory are not incorporated into the systematics of the genus, but the various species are separated on the characteristics of the types found in nature. There seems no reason why a similar attitude should not be adopted in the case of *Fusarium*. It is possible, of course, that some members of the genus would be found to display a wide range of variability in the field, and if so, such organisms would present difficulties. It may be concluded, however, that in systematic studies of Fusaria, close attention should be paid to the degree of variation displayed *in nature*, the mutants that arise under the highly abnormal conditions of artificial culture providing no sound basis for the broadening of species descriptions.

Taxonomic Characters in the Section Elegans

Since the wide range of variability shown by the Elegans Fusaria in culture renders it advisable that only the original types be considered in a taxonomic treatment, it is important to know whether these are sufficiently distinct to permit separation on morphological grounds. While the Elegans Fusaria studied by the writer were closely similar in certain cases, in others differences were consistently apparent, and these were investigated to determine to what extent they would permit a convenient morphological separation of the organisms concerned.

The size and shape of conidia have hitherto been regarded as highly important taxonomic characters in *Fusarium*. All the Elegans Fusaria studied here produced mainly microconidia on potato dextrose agar. But Wollenweber *et al.* (15) do not regard these spores as of much diagnostic value except in certain cases where they have definite characters such as pear-like shape or formation in chains. Since it is not permissible to employ the macroconidia that often appear abundantly in patch mutants, it follows that one should either measure and compare the size and shape of the microconidia, or search for a medium on which the wild type mycelium will produce macroconidia.

To investigate the former alternative, an experiment was performed with five Elegans Fusaria to determine to what extent these are separable by the characteristics of the microconidia produced on potato dextrose agar. The muskmelon, tomato, and navy bean Fusaria, and Soil Fusaria *A* and *B* were grown in the same Petri dishes, which were kept at 27° C. in an incubator inside which the humidity was maintained at a fairly high level by strips of blotting paper, the lower ends of which were immersed in water. From each of the wild types, 100 spores were measured at intervals of 4, 8, 12, 16, and 26 days after the plates were inoculated; the results of the experiment are summarized in Table I.

Considering first the effect of age of culture on spore size, it is noted that in all five organisms the tendency is for spore size to decrease as the cultures age. Two explanations may be advanced to account for this. The spores may actually shrink through loss of water or smaller spores may be cut off by the conidiophores as the supply of nutrient in the medium became exhausted. It is worth noting in this connection that all the conidia produced by a single conidiophore are not identical in size but show about as much variation as that existing between spores from different conidiophores. It was possible to make this observation since the spores from individual conidiophores tended to cling together in small groups. In view of the progressive decrease in average size of the spores, it would seem wise to decide upon an arbitrary age of cultures at which measurements should be made. Since on the whole the rate of decrease was greatest during the first 16 days, after which the average size tended to remain relatively constant, two weeks would perhaps be a suitable age for the taking of spore measurements.

It will be apparent from the table that the spore sizes fall into two well-defined groups. The spores of the first group, which includes the muskmelon *Fusarium* and Soil Fusarium *B*, are longer than those of the navy bean and tomato Fusaria and Soil Fusarium *A*, which comprise the second group. This difference affects the ratio of width/length, causing it to be lower in the first group. Within the groups differences do not seem as apparent. In the second group, for instance, the tomato *Fusarium* conidia seem on the average shorter than those of either the navy bean *Fusarium* or Soil Fusarium *A*. In the first group the spores of the muskmelon *Fusarium* seem slightly

TABLE I

COMPARISON OF THE DIMENSIONS OF THE NONSEPTATE CONIDIA PRODUCED BY FIVE FUSARIUM WILD TYPES ON POTATO DEXTROSE AGAR. EACH AVERAGE INVOLVES 100 CONIDIA

Organism	Age of culture, days	Maximum (μ)	Minimum (μ)	Average (μ)	Ratio: $\frac{\text{Width}}{\text{Length}}$
Muskmelon *Fusarium*	4	13.3 × 3.6	5.5 × 2.4	8.68 × 3.15	0.36
	8	12.4 × 3.1	5.5 × 2.7	8.02 × 3.01	0.38
	12	13.8 × 3.6	5.8 × 2.7	8.36 × 3.09	0.37
	16	10.9 × 2.7	4.7 × 2.5	7.45 × 3.06	0.41
	26	10.5 × 3.5	4.9 × 2.7	7.49 × 2.97	0.40
Tomato *Fusarium*	4	11.3 × 3.6	4.0 × 2.9	6.45 × 3.13	0.49
	8	8.3 × 3.3	4.0 × 2.0	5.65 × 2.97	0.53
	12	7.8 × 3.5	4.4 × 2.5	5.97 × 3.19	0.54
	16	8.7 × 4.0	3.8 × 2.9	5.88 × 3.13	0.53
	26	10.2 × 3.8	4.2 × 2.5	5.70 × 3.04	0.53
Navy bean *Fusarium*	4	10.7 × 3.6	4.0 × 2.9	6.77 × 3.13	0.46
	8	9.3 × 3.6	3.6 × 2.2	6.07 × 3.09	0.51
	12	8.4 × 3.6	4.4 × 1.8	5.90 × 2.89	0.49
	16	9.5 × 3.6	4.2 × 2.2	6.18 × 2.96	0.48
	26	9.8 × 3.5	4.4 × 2.4	5.98 × 2.78	0.47
Soil Fusarium *A*	4	14.6 × 3.6	4.5 × 2.5	8.79 × 3.29	0.38
	8	11.3 × 3.6	4.0 × 2.7	6.88 × 3.36	0.49
	12	12.0 × 4.0	4.4 × 2.9	6.82 × 3.27	0.48
	16	10.4 × 4.0	4.0 × 2.5	5.94 × 3.09	0.52
	26	10.6 × 4.4	4.0 × 2.4	6.11 × 3.13	0.51
Soil Fusarium *B*	4	12.2 × 3.3	5.5 × 2.2	8.54 × 2.87	0.34
	8	13.5 × 4.0	5.8 × 2.2	7.94 × 2.76	0.35
	12	10.9 × 2.9	5.5 × 2.2	7.62 × 2.86	0.38
	16	13.1 × 4.0	5.5 × 1.8	7.99 × 2.94	0.37
	26	11.3 × 3.1	4.5 × 1.8	7.49 × 2.68	0.36

wider than those of Soil Fusarium *B*, which is reflected in the ratio of width/length, but these separations are neither as sharp nor as consistent as those existing between the two main groups. However, in the latter an additional feature of the spores may be employed to effect a separation, since the conidia of Soil Fusarium *B* were often narrower at one end. In addition, in Soil Fusarium *B* about 2% of the conidia were septate, having one, two, or even three septa. Septate spores were very rare in the other four Fusaria on this medium. The conclusion seems reasonable that, while in many instances a separation may be effected on the basis of spore measurements, it will often be necessary to employ further characters either of the spores or of cultures to distinguish the various members of the section Elegans.

Considering first, in this connection, the spores produced on potato dextrose agar, characters other than size that should be of importance are shape and abundance. The value of a characteristic shape has been indicated above with Soil Fusarium *B*. With regard to abundance, when a small amount of aerial mycelium from the tomato *Fusarium* is placed in a drop of water

on a slide and flattened with a coverslip, a marked cloudiness is noted, which proves to be caused by the enormous number of microconidia borne on the mycelium. This effect was not obtained with the muskmelon *Fusarium*. Soil Fusarium *B* resembled the muskmelon *Fusarium*, while Soil Fusarium *A* and the navy bean *Fusarium* sporulated very abundantly though they did not seem to equal the tomato *Fusarium* in this respect.

It was observed that when test-tubes of moist sterilized soil were inoculated with the various Elegans Fusaria studied here, a small amount of loose aerial mycelium appeared above the surface of the soil. On this mycelium spores were borne that varied from small non-septate to large several-septate conidia. Since the macroconidia here are produced by the wild type, there would be no objection to employing them taxonomically. Some differences were apparent, the macroconidia of the muskmelon *Fusarium* being longer and less pointed at the ends than those of the navy bean *Fusarium*, but no experiment was performed to investigate these differences more fully. The indication is, however, that features displayed by the wild types on media other than potato dextrose agar should be of taxonomic value.

The general appearance in culture, including the appearance of the aerial mycelium and the pigmentation of the immersed mycelium, differs in many cases. The former, however, is always difficult to describe in words and the latter was found to be very unreliable, varying even with different lots of the same type of medium.

The 'sclerotia' that often appear in cultures of Fusaria have already been discussed (5). Not all the Fusaria studied here seemed to produce these but in cultures of the muskmelon, pea, and soybean Fusaria, and Soil Fusarium *A* they were abundant, as many as 200 appearing in a single Petri dish culture. It must be realized that since these appeared to be of mutant origin, indicating genetic instability in the organisms that produce them, it may actually be the latter character that is being compared, but there seems no reason why the mutation frequency of a given organism on a given medium should not be treated as a taxonomic character. Likewise, the frequency and general appearance of patch mutants should not be overlooked in a taxonomic study. Patch mutants were very abundant and conspicuous (on potato dextrose agar) in the muskmelon, pea, cabbage, and flax Fusaria.

The taxonomic value of the phenomenon termed 'cultural interaction' has been discussed in a preceding publication (5), where the conclusion was reached that it is sufficiently specific to be an aid both in diagnosing Fusaria and in providing a check on diagnoses arrived at by other criteria, particularly in the case of saprophytes.

The evidence thus indicates that characters of taxonomic value, both morphological and physiological, are by no means infrequent in the wild types of Elegans Fusaria. It is hoped that the present studies will lead to the establishment of a morphological classification that will avoid confusion by adhering strictly to the wild types and that may be correlated with pathogenicity trials in the case of parasites.

An indication of the way in which this may be approached is provided by the key that follows. It will be noted that a final separation is not effected in certain cases, but perhaps the characters expressed on other media would help. Spore measurements may also prove useful here. With the cotton and cowpea Fusaria a very sharp separation is provided by cultural interaction as was shown in the preceding paper of this series (5). 'Type' organisms would be useful in interpreting keys of this nature. The muskmelon and tomato Fusaria could be employed in this respect, the former exemplifying sparse sporulation and the abundant and conspicuous production of patch mutants and sclerotia, and the latter exemplifying profuse sporulation and infrequent production of patch mutants and sclerotia. It seems apparent that much careful taxonomic work is required before an accurate classification of this section, and perhaps of others as well, may be drawn up.

Morphological Key to 13 Elegans Fusaria Based on the Features Expressed on Potato Dextrose Agar

A. Sporulation on aerial mycelium sparse
  B. Sclerotia abundant ..... Muskmelon *Fusarium*
  BB. Sclerotia very few or absent
    C. Patch mutants abundant and conspicuous
      D. Patch mutants appear as white, cottony areas in aerial mycelium
        Flax *Fusarium*
      DD. Patch mutants appear white in aerial mycelium but are dense and extend into the immersed mycelium ..... Cabbage *Fusarium*
    CC. Patch mutants infrequent ..... Soil Fusarium *B*

AA. Sporulation on aerial mycelium profuse
  B. Sclerotia abundant
    C. Plectenchymatic patch mutants present
      D. Sclerotia irregularly distributed through medium .... Soil Fusarium *A*
      DD. Sclerotia regularly distributed in the sense that they are either at the surface of the medium or are deeply sunken in the medium, adjacent to glass of Petri dish ..... Soybean *Fusarium*
    CC. Plectenchymatic patch mutants very few or absent ..... Pea *Fusarium*
  BB. Sclerotia very few or absent
    C. Plectenchymatic patch mutants present ..... Aster *Fusarium*
      Navy bean *Fusarium*
      Gladiolus *Fusarium*
    CC. Plectenchymatic patch mutants very few or absent
      D. Mottled appearance developing after two or three weeks in the immersed mycelium ..... Cotton *Fusarium*
        Cowpea *Fusarium*
      DD. Mottled appearance not developing in the immersed mycelium
        Tomato *Fusarium*

Discussion

It seems apparent that a great deal of confusion has arisen in the taxonomy of the genus *Fusarium* as a consequence of mutation in pure culture. Snyder and Hansen (12) describe many instances of apparent transmutation of species through variation. Padwick (6) found that duplicate tubes of various

Fusarium cultures varied greatly in the ratios of continuous to septate spores. In fact, his very thorough taxonomic work on part of the section Elegans led him to state: "We are forced to the conclusion that the cultures of *Orthocera-Fusaria* maintained at Baarn cannot, with one or two exceptions, be recognized from the descriptions given by Wollenweber and Reinking." The work of the present writer (3, 4, 5) has confirmed the observations of Snyder and Hansen and of Padwick in that variants widely different from the original were obtained in culture, and a number of generalizations seem deducible from this work that are of significance with regard to the taxonomy of the genus. (1). In most cases the initial isolates of a given *Fusarium* are identical, or closely similar, in culture. (2). When grown in pure culture on a laboratory medium such as potato dextrose agar, the original or wild type is soon displaced by mutants that are more successful than the 'parent' in this highly abnormal environment. (3). The process seems unidirectional, no instance of reversion having been noted after a given mutant was purified by the single-spore method. It would thus seem advisable to base the taxonomy of the genus on fresh isolates of the organisms, unless particular care is taken to maintain these in the pure state. Otherwise there appears little hope that a usable morphological treatment of *Fusarium* will be developed.

Snyder and Hansen (12) in discussing the Wollenweber and Reinking classification (14) make the statement: "Another serious breakdown in the system occurs when fungi which originally may have been specifically identified are re-examined and re-identified after a period in culture, for then it may be found that the first and second identifications do not agree, perhaps not even in respect to section characters". This is an agreement with the foregoing generalizations. However, the conclusion drawn by Snyder and Hansen, that this situation necessitates the merging of species and abandonment in many instances of all hope of morphological separation does not necessarily follow, since it must first be proved that the process occurs in nature as well as in artificial culture. Padwick (7) has criticized the action of Snyder and Hansen in converting the section Elegans into a single species. He states that they have reduced the possibility of making errors by the simple procedure of being vague. Additional reasons given for not accepting this revision are (1) that Snyder and Hansen assume Wollenweber and his associates to be wrong in their grouping of isolates into species, yet right in their grouping of species into sections, and (2) they tend to lose objectivity by concentrating on the potentialities of the species in test-tubes rather than on its objective existence in nature. To clarify the taxonomic situation in *Fusarium*, Padwick proposes the concept of "focal point species", which involves the study of Fusaria in their natural habitat. This appears similar to the 'wild type' concept of the present writer, and some such revision of the basis for taxonomy in the genus would seem advisable.

It is conceivable that the objections raised in the present series with regard to both prevailing systems of Fusarium taxonomy may lead to a reconciliation between the two. Possibly a system based on morphology would be to a

large extent successful and useful, provided only the wild types are included. But where differences are very slight, or else are confused by the presence in nature of a number of cultural strains, the merging of species as proposed by Snyder and Hansen would be desirable.

Acknowledgments

The writer wishes to express his sincere thanks to Dr. L. W. Koch, Officer-in-Charge of the Dominion Laboratory of Plant Pathology, Harrow, Ont., for rendering available the facilities for this work and for his constant interest during the course of the investigations. The contributions of diseased specimens and cultures have greatly increased the scope and value of these studies, and the writer is very grateful to those who assisted in this regard. Thanks are due to Professor D. L. Bailey of the Department of Botany, University of Toronto, and to Drs. L. W. Koch and A. A. Hildebrand of the Dominion Laboratory of Plant Pathology, Harrow, for valuable suggestions both during these studies and following the preparation of the manuscript in its initial form.

References

1. APPEL, O. and WOLLENWEBER, H. W. Grundlagen einer Monographie der Gattung *Fusarium* (Link). Arb. Kaiserl. biol. Anstalt Land-Forstw. 8 : 1-207. 1910.
2. BROWN, W. Studies in the genus *Fusarium*. VI. General description of strains, together with a discussion of the principles at present adopted in the classification of *Fusarium*. Ann. Botany, 42 : 285-304. 1928.
3. MILLER, J. J. Studies on the *Fusarium* of muskmelon wilt. I. Pathogenic and cultural studies with particular reference to the cause and nature of variation in the causal organism. Can. J. Research, C, 23 : 16-43. 1945.
4. MILLER, J. J. Studies on the *Fusarium* of muskmelon wilt. II. Infection studies concerning the host range of the organism and the effect of environment on disease incidence. Can. J. Research, C, 23 : 166-187. 1945.
5. MILLER, J. J. Cultural and taxonomic studies on certain Fusaria. I. Mutation in culture. Can. J. Research, C, 24 : 188-212. 1946.
6. PADWICK, G. W. The genus *Fusarium*, III. A critical study of the fungus causing wilt of gram (*Cicer arietinum* L.) and of the related species in the Sub-section Orthocera. Indian J. Agr. Sci. 10 : 241-284. 1940.
7. PADWICK, G. W. The genus *Fusarium*, VI. A recent attempt at mass revision. Indian J. Agr. Sci. 11 : 663-674. 1941.
8. REINKING, O. A. and WOLLENWEBER, H. W. Tropical Fusaria. Philippine J. Sci. 32 : 103-253. 1927.
9. SHERBAKOFF, C. D. Fusaria of potatoes. N.Y. Cornell Agr. Expt. Sta. Memoirs, 6 : 89-270. 1915.
10. SNYDER, W. C. and HANSEN, H. N. The species concept in *Fusarium*. Am. J. Botany, 27 : 64-67. 1940.
11. SNYDER, W. C. and HANSEN, H. N. The species concept in *Fusarium* with reference to section *Martiella*. Am. J. Botany, 28 : 738-742. 1941.
12. SNYDER, W. C. and HANSEN, H. N. The species concept in *Fusarium* with reference to *Discolor* and other sections. Am. J. Botany, 32 : 657-666. 1945.
13. WOLLENWEBER, H. W. Identification of species of *Fusarium* occurring on the sweet potato, *Ipomoea batatas*. J. Agr. Research, 2 : 251-286. 1914.
14. WOLLENWEBER, H. W. and REINKING, O. A. Die Fusarien. Paul Parey, Berlin. 1935.
15. WOLLENWEBER, H. W., SHERBAKOFF, C. D., REINKING, O. A., JOHANN, H., and BAILEY, A. A. Fundamentals for taxonomic studies of *Fusarium*. J. Agr. Research, 30 : 833-843. 1925.

THE MICROFLORA OF STORED WHEAT[1]

By Norman James[2], Joyce Wilson[3], and Egon Stark[4]

Abstract

Wheat passing through the Winnipeg market in 1943 and 1945 harboured a large number of micro-organisms. They were not found on all kernels, but were present on all 10-gm. samples. The number was smallest on No. 1 Manitoba Northern red spring wheat and progressively larger on each successive lower grade.

The organisms consisted chiefly of two species of bacteria, which persisted on the grain even after repeated vigorous washings. One was identified as *Bacterium herbicola aureum* Duggeli. The other undoubtedly is a representative of the genus *Pseudomonas*. It is suggested that these species should be considered epiphytic on wheat.

Introduction

Although grain would be expected to harbour bacteria, yeasts, and fungi that might affect its keeping quality, little information on the saprophytic flora of stored wheat is available. Duggeli (1904 (5, 6)) reported that healthy grain kernels harboured on their surfaces very large numbers of bacteria belonging mainly to one species. Morgenthaler (1918 (12)) found bacteria ranging in numbers from 80,000 to 25,000,000 per gm. on normal or healthy wheat; and fungi ranging from 4000 to 7200 per gm. He claimed that healthy kernels were free from fungi and that injury to the seed coat of some kernels was responsible for the small fungal population on the weighed sample. Mack (1936 (10)) counted the bacteria on sprouted and unsprouted wheat. The number of bacteria on 20 kernels of unsprouted wheat averaged 60,000 per kernel; and on 20 kernels of sprouted wheat 1,300,000 per kernel.

Kent-Jones and Amos (1930 (8)) made estimates of bacteria on 21 samples of wheat passing through the normal channels of trade in Great Britain. Estimates from nutrient agar plates, incubated at 37° C. for 48 hr., ranged from 8000 to 8,000,000 per gm. of wheat.

Gustafson and Parfitt (1933 (7)) reported counts ranging from 46,000 to 3,260,000 per gm. on 19 samples of wheat investigated.

The significance of the flora on wheat is of interest at this time because of the somewhat general belief that micro-organisms cause the heating of grain under certain conditions of storage (9, 11, 14) and because of the large surplus of wheat stored in elevators during the past decade.

This report deals with isolations of bacteria, yeasts, and filamentous fungi from different grades of stored wheat by the technique of the bacteriologist interested in the general, rather than the pathogenic, flora of grain.

[1] *Manuscript received May 3, 1946.*

Contribution from the Department of Bacteriology and Animal Pathology, The University of Manitoba, with financial assistance from the National Research Council of Canada. Published as Paper No. 244 of the Associate Committee on Grain Research.

[2] *Professor of Bacteriology.*

[3] *Formerly Research Assistant.*

[4] *Research Assistant.*

Samples and Methods

Samples of four grades of Manitoba Northern red spring wheat and of No. 5 and No. 6 wheat were supplied through the courtesy of the Chemist-in-Charge, Grain Research Laboratory, Board of Grain Commissioners for Canada. They represented composites of all wheat of these grades passing through the regular channels of trade in Winnipeg in certain two week periods in the summer of 1943. Each sample consisted of about 150 gm. Tests were made on six replicate 10 gm. portions of each sample. Each portion was transferred aseptically to a 6 oz. screw-top bottle containing 100 ml. of sterile water and about 10 gm. sterile gravel and was shaken for 30 min. on a to-and-fro shaking machine. Appropriate dilutions were made; and duplicate plates were prepared in a special transfer room previously sprayed with a disinfecting solution. Standard nutrient agar was used as a substrate for bacteria; and Czapek's agar, plus 0.5 ml. of 10% lactic acid per 100 ml. of medium, for yeasts and fungi. Plates were incubated at 26° C. for six days. Counts were recorded as numbers of bacteria, yeasts, and fungi in the washings from 1 gm. of wheat. The experiment was carried out four times in 1943 and repeated four times in 1945.

Numbers of Organisms in First Washings from Wheat

The bacterial populations in the washings from the samples studied in 1943 were found to be extremely high, ranging from 280,000 to 164,000,000 per gm. of wheat; and the increase in numbers from Grade 1 to Grade 6 was strikingly regular in each replication of the experiment. Yeasts, likewise, were present in large numbers—6200 to 64,000; but not all replications showed the same trend in numbers from Grade 1 to Grade 6. On the contrary, only relatively small numbers of fungi—420 to 1870—were found in the washings; and the difference between grades appeared to show little consistency.

The trials carried out in 1945 showed essentially the same trends.

The results of the four replications of the experiment in 1943, expressed as averages of logarithms of counts, and of the four replications in 1945 are shown graphically in Fig. 1.

Since the percentage of frosted, immature, and weathered kernels is considered in establishing the grade of wheat, it appeared advisable to determine to what extent the populations on individual kernels of these types might account for the population differences in the different grades referred to in the previous experiment. From a composite sample of No. 4 Manitoba Northern red spring wheat 30 kernels of each of the following types were selected—vitreous, weathered, frosted, and immature.* Each kernel was tested by the procedure outlined under Samples and Methods, with one exception. Ten ml. of sterile water was used, instead of 100 ml., in the initial dilution.

* *Dr. P. J. Olson and Mr. A. J. Lejeune of the Department of Plant Science made the selection.*

In these trials, too, bacterial counts showed the widest range. On the average, vitreous kernels harboured the smallest numbers, with weathered, immature, and frosted kernels sustaining progressively increasing numbers of

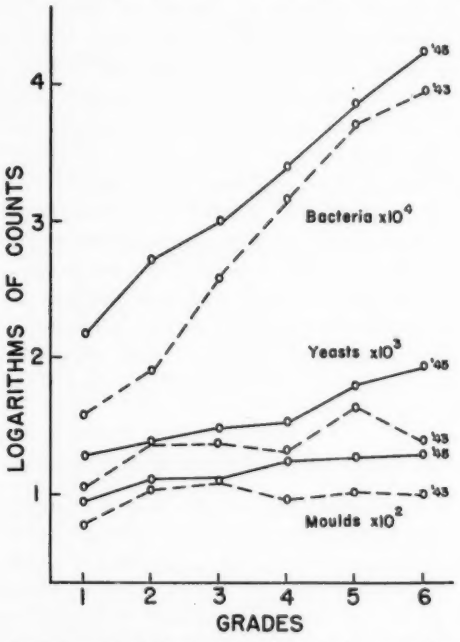


FIG. 1. *Relation between grades of wheat and numbers of micro-organisms in first washings; averages of 24 determinations.*

bacteria. The results on the four types, expressed as logarithms of averages from 30 kernels in each case, are shown graphically in Fig. 2. Some kernels of each type gave counts that did not conform with the general trend illustrated in Fig. 2. This is brought out in Fig. 3*a*, which shows the distribution of the kernels in classes based on logarithms of numbers on separate kernels. Plate counts of less than 100,000 bacteria per kernel were produced by 28 vitreous kernels whereas only 27 weathered, 19 immature, and 18 frosted kernels fell in classes under 100,000.

The yeast population on the same kernels failed to show a consistent relation to type of kernel, even though the trend for yeasts in Fig. 2 appears to indicate it. One immature kernel harboured a larger number of yeasts than the total on the other 29, which accounts for the high point for immature kernels in the figure. The class distribution of kernels based on yeast counts, shown in Fig. 3*b*, makes it apparent that the yeast population was highly variable. The fungal population on these kernels was small and failed to indicate a consistent difference between types of kernel, as is shown in Figs. 2 and 3*c*.

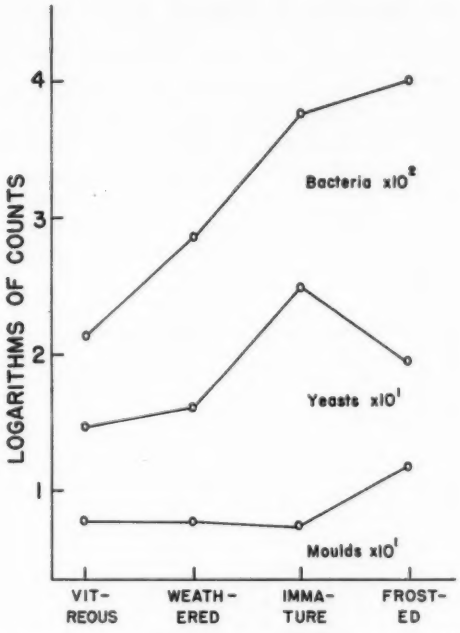


FIG. 2. *Relation between types of wheat kernel and numbers of micro-organisms; averages of 30 kernels.*

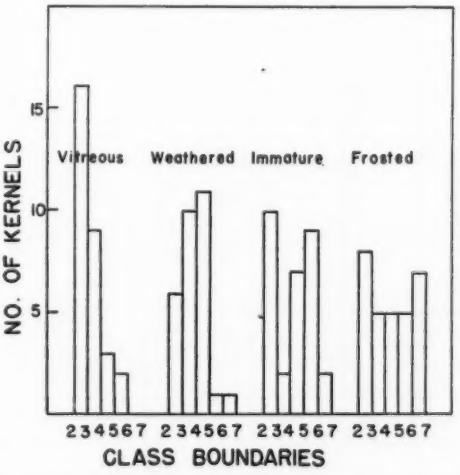


FIG. 3a. *Distribution of different types of wheat kernel based on logarithms of counts of bacteria.*

Numbers of Organisms in Twelfth Washings from Wheat

This part of the study was carried out to determine the numbers of organisms on wheat after repeated washings in sterile water. Each of six 10 gm. portions from samples of each of the six grades of wheat was exposed to 12 consecutive washings in sterile water and gravel as follows: five washings for 30 min. on

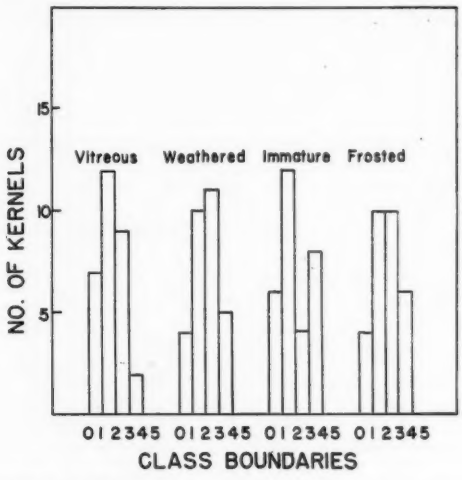


FIG. 3b. *Distribution of different types of wheat kernel based on logarithms of counts of yeasts.*

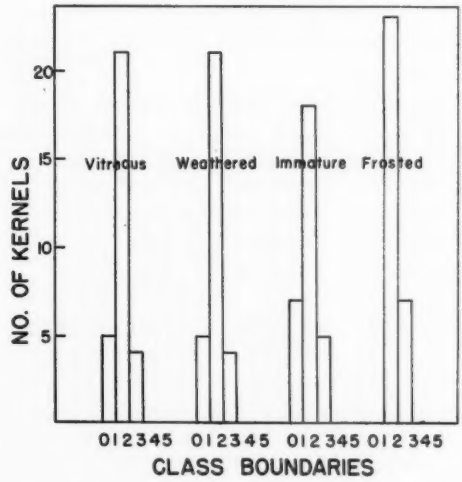


FIG. 3c. *Distribution of different types of wheat kernel based on logarithms of counts of fungi.*

the mechanical shaker, six rinsings, and a final washing for a 30 min. period. Appropriate dilutions for plating were made from the final wash water. The experiment was carried out twice.

The numbers of bacteria in the final wash waters were found to be high—about 20% of those found in the first washings from the samples used in the first experiment. Again, the increase from No. 1 to No. 6 grade was regular. Yeasts and fungi also were present in the final wash waters, but in relatively small numbers. The results of the two replications of this experiment are shown in Fig. 4. They present essentially the same picture as is shown for the first washings from wheat in Fig. 1.

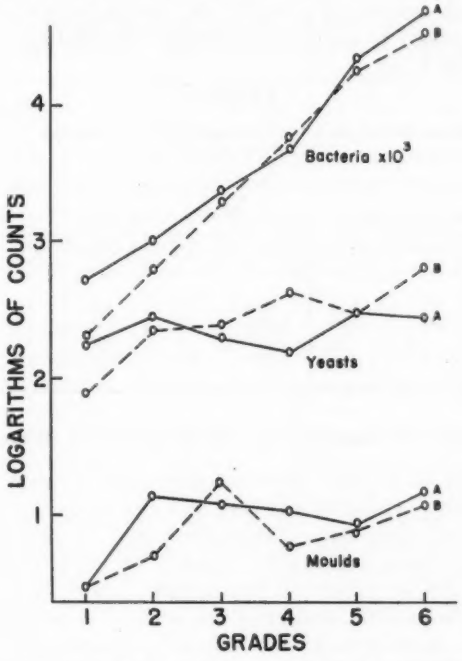


FIG. 4. *Relation between grades of wheat and numbers of micro-organisms in twelfth washings; averages of six determinations.*

In order to obtain direct evidence on the proportion of organisms remaining on wheat after vigorous washings, counts were made on the first and twelfth washings from a sample of No. 1 Manitoba Northern and a sample of No. 4 Manitoba Northern. The experiment was replicated three times and the averages used to indicate the proportion of the various groups of organisms in the twelfth washings. In the No. 1 grade sample, the counts in the twelfth washings were, for bacteria, 21%; for yeasts, 4%; and for fungi, 33% of the counts in the first washings; and in the No. 4 grade sample they were 37%,

3%, and 19%, respectively. It should be noted, however, that the fungal populations in the first washings were small—1400 per gm. in the No. 1 grade sample and 2900 in the No. 4 grade sample.

Numbers of Organisms on Crushed Wheat After 12 Washings

If such large numbers of bacteria and yeasts are to be found in all washings from wheat it is evident that there must be large numbers of organisms that cannot be removed by washing. In order to get information on this point four portions of a sample of No. 1 Manitoba Northern wheat were exposed to 12 washings as outlined previously. Then after draining off the twelfth water each sample was ground with a pestle and mortar, under aseptic conditions, and raised to appropriate dilutions for plating. The results are presented in Table I.

TABLE I

NUMBERS OF ORGANISMS IN WASHINGS FROM WHEAT AND IN THE CRUSHED RESIDUE (PER GRAM)

Replicate portions	Bacteria $\times 10^3$			Yeasts $\times 10^1$			Fungi $\times 10^1$		
	1st	12th	Residue	1st	12th	Residue	1st	12th	Residue
1	108	21	630	760	10	220	15	15	60
2	102	98	120	1020	15	230	32	42	10
3	43	1.7	49	430	3	370	13	1.5	1.5
4	104	7.7	144	455	12	273	16	1.5	50

Types of Bacteria in Washings from Wheat

Identification of all bacteria on wheat presents a problem that involves a great deal of study. Probably the best that can be hoped for is to confine the undertaking to certain types that predominate. A careful examination of colony types on plates repeatedly revealed the fact that the majority of the colonies fell in either of two groups. This was most evident on plates from the twelfth washings from wheat. Accordingly, an attempt was made to get an estimate of the relative prevalence of these two types and to identify them. For the first test all the 99 colonies developing on two plates from a one-millionth dilution of the twelfth washing from a sample of No. 6 wheat were picked into nutrient broth. After incubation each culture was examined microscopically for evidence of purity and picked to an agar slant medium for later studies. On the basis of this preliminary study, involving growth characteristics on nutrient broth and on nutrient agar, shape, size, motility, and Gram's staining reaction, 30% of the isolations proved to be of one type and 45% to be of another type, different in several respects from the former. These were designated Types *A* and *B* and will be referred to hereafter by these letters. A second test, replicated three times on a sample of No. 1 Manitoba Northern red spring wheat, gave an

average incidence of 34% for Type *A* and of 30% for Type *B;* and a third test on No. 4 Manitoba Northern gave average incidences of 37% and 40%, respectively. The remaining 23 to 36% of the colonies developing on the plates used represented what were accepted to be different types, or in some cases mixed cultures. They were not considered further.

A representative culture of each of Type *A* and Type *B* was selected, replated through two successive platings and isolations to ensure purity and investigated in detail by recognized pure culture study methods.

Type *A* undoubtedly represents a species reported by Burri (1903 (4)) to be an epiphyte normally found on the leaves of a wide variety of plants. Duggeli (1904 (5)) found the same species on a variety of seeds, including common and durum wheats. He claimed that it represented from 97 to 100% of the flora on five samples of wheat investigated. In a later report (1904 (6)) Duggeli described the species in detail and named it *Bacterium herbicola aureum.* He stressed the formation of characteristic zooglea, which could be observed as sausage-like clumps under low magnification, a phenomenon that was apparent in our isolations only after careful examination at high magnification. Mack (1936 (10)) studied several strains of the species in detail. She claimed that it should be called *Flavobacterium herbicola*, and not *Pseudomonas trifolii* as listed in the fifth edition of Bergey's manual (1939 (1)).

Type *B*, which appeared about as often as Type *A* in our isolations, evidently was not observed by Burri or Duggeli. It could be distinguished readily from the latter type by the lack of yellow pigment on surface colonies and its strong fluorescence in sucrose broth when exposed in ultra-violet light. Duggeli claimed that *Bacterium fluorescens* (*Pseudomonas fluorescens*) represented the second most prevalent species on some seeds and seedlings, but in relatively small numbers. Undoubtedly, Type *B* is a representative of the genus *Pseudomonas* that does not belong to any of the species listed in Bergey's manual. It differs from *Pseudomonas fluorescens* in its strong tendency to slime formation, its failure to develop colour on nutrient agar or in nutrient gelatine or nutrient broth, and its failure to reduce nitrates or to produce acid from dextrose.

Types of Fungi in Washings from Wheat

A preliminary study of the types of fungi developing on Czapek's agar from the first washings from the samples investigated in 1943 was carried out.* This involved selection of typical colonies from a large number of plates, sub-culturing on Czapek's agar slants, and identification studies, without attempting to obtain detailed information on the distribution of the types isolated. Species of *Penicillium* predominated, with *P. chrysogenum*, *P. frequentans* and *P. terrestre* occurring the most frequently. Species of *Aspergillus*, *Alternaria*, *Fusarium*, and *Cladosporium*, likewise, represented a large

* *This part of the study was carried out by Miss Wilson under the guidance of Dr. H. J. Brodie of the Department of Botany.*

proportion of the cultures developing on the plates. The following types were identified: *Acrostalagmus cinnabarinus* Corda; *Alternaria tenuis* auct. sensu Wiltshire; *Aspergillus candidus* Link; *A. flavus* Link; *A. fumigatus* Fres.; *A. glaucus* Link; *A. niger* van Tiegh.; *A. Oryzae* (Ahlburg) Cohn; *A. versicolor* (Vuill.) Tirab.; *Botrytis cinerea* Pers.; *Cephalosporium curtipes* Sacc.; *Cephalosporium* spp.; *Cephalothecium roseum* Corda; *Cladosporium herbarum* (Pers.) Link; *Fusarium culmorum* (W. G. Smith) Sacc.; *F. Poae* (Peck) Wollenw.; *F. Scirpi* var. *acuminatum* (Ell. and Ev.) Wollenw.; *F. semitectum* Berk. and Rav. var. *majus* Wr.; *Helminthosporium sativum* Pam., King, and Bakke; *Hormodendron pallidum* Oudem.; *H. viride* (Fres.) Sacc.; *Monilia* spp.; *Mucor circinelloides* van Tiegh.; *M. racemosus* Fres.; *Paecilomyces varioti* Bain.; *Penicillium chrysogenum* Thom; *P. flavi-dorsum* Biourge; *P. frequentans* Westl.; *P. purpurogenum* Stoll; *P. rugulosum* Thom; *P. spinulosum* Thom; *P. terrestre* Jensen; *Phoma* spp.; *Rhizopus* spp.; *Scopulariopsis brevicaulis* (Sacc.) Bain.; *Scopulariopsis* sp.; *Septoria nodorum* Berk.; *Torula* spp.; *Trichoderma lignorum* (Tode) Harz.

All except five of the species listed above have been isolated from Manitoba soils (2, 3). The additional species obtained from wheat follow: *Cephalothecium roseum; Septoria nodorum; Fusarium semitectum; Aspergillus Oryzae,* and the *A. glaucus* group.

Seven of the species isolated in this study are listed in Orton's bibliography (1931 (13)) on seed-borne parasites. These follow: *Alternaria tenuis; Aspergillus niger; Cephalothecium roseum; Cladosporium herbarum; Fusarium culmorum; Helminthosporium sativum;* and *Septoria nodorum.*

Discussion

The finding of such large numbers of certain species of bacteria on wheat grains points to the existence of a flora that may be termed epiphytic. As suggested earlier by Burri (4), with respect to the flora on the leaves of plants, these bacteria cannot be considered mere chance contaminants that reach the grain through air currents, insects, or other means, but must represent primarily types that develop on it. Evidently this flora is not present on every kernel, but is harboured by a sufficiently large number of them to account for the enormous population on all 10-gm. samples studied. Likewise, it is apparent that their presence cannot be attributed to any known defect in the kernel. They should be considered commensals developing on the seed-coat or in its intercellular spaces. They must be able to proliferate under the conditions of grain storage or, at least, withstand such conditions for long periods of time. What their role is in the production of the characteristic properties of cereal products or in the development of certain defects in them is not known.

Since yeasts are distributed widely in nature, particularly on fermentable materials, their presence on wheat grains might have been anticipated. The evidence of an epiphytic yeast flora on wheat is convincing since particles of soil or dust on the kernel would not be expected to harbour many yeasts. In

fact, only rarely have yeasts been found developing on Czapek's medium when soil is plated at the dilution used in this study (2, 3). However, since this epiphytic yeast flora represents relatively small numbers, which fluctuate erratically from sample to sample, it is probable that it has little practical importance.

The fungal population, on the contrary, scarcely can be considered epiphytic. It is small at the moisture level of stored wheat, which may be accepted as indicating that this population is not the result of proliferation. However, it is persistent, which probably means that spores are held mechanically and protected in the crease of the kernel or in crevices of the injured seed coat in some cases. In general, it represents species that occur commonly in soil and dust in large numbers.

References

1. BERGEY, D. H. *et al.* Manual of determinative bacteriology. 5th ed. The Williams and Wilkins Co., Baltimore. 1939.
2. BISBY, G. R., JAMES, N., and TIMONIN, M. Fungi isolated from Manitoba soil by the plate method. Can. J. Research, 8 : 253-275. 1933.
3. BISBY, G. R., TIMONIN, M. I., and JAMES, N. Fungi isolated from soil profiles in Manitoba. Can. J. Research, C, 13 : 47-65. 1935.
4. BURRI, R. Die Bakterienvegetation auf der Oberflache normal entwickelter Pflanzen. Zentr. Bakt. Parasitenk. Infekt. II Abt. 10 : 756-763. 1903.
5. DUGGELI, M. Die Bakterienflora gesunder Samen und daraus gezogener Keimpflanchen. Zentr. Bakt. Parasitenk. Infekt. II Abt. 12 : 602-614. 1904.
6. DUGGELI, M. Die Bakterienflora gesunder Samen und daraus gezogener Keimpflanchen. Zentr. Bakt. Parasitenk. Infekt. II Abt. 13 : 56-63 and 198-207. 1904.
7. GUSTAFSON, C. B. and PARFITT, E. H. Effect of numbers of bacteria on the development of rancidity in soft wheat flour. Cereal Chem. 10 : 233-238. 1933.
8. KENT-JONES, D. W. and AMOS, A. J. Preliminary studies in the bacteriology of wheat and flour. Analyst, 55 : 248-268. 1930.
9. LEACH, W. Studies on the metabolism of cereal grains. III. The influence of atmospheric humidity and mould infection on the carbon dioxide output of wheat. Can. J. Research, C, 22 : 150-161. 1944.
10. MACK, E. Untersuchungen über *Bacterium herbicola*. Zentr. Bakt. Parasitenk. Infekt. II Abt. 95 : 218-261. 1936.
11. MILNER, M. The respiration and storage behaviour of soybeans. PhD. Thesis. University of Minnesota. 1945.
12. MORGENTHALER, O. Uber die Mikroflora des normalen und muffigen Getreides. Landw. Jahrb. Schweiz. 32 : 548-570. 1918.
13. ORTON, C. R. Seed-borne parasites. A bibliography. W. Virginia Univ. Agr. Expt. Sta. Bull. 245. 1931.
14. SALLANS, H. R., SINCLAIR, G. D., and LARMOUR, R. K. The spontaneous heating of flaxseed and sunflower seed stored under adiabatic conditions. Can. J. Research, F, 22 : 181-190. 1944.

Imperial Agricultural Bureaux

This organization set up by the Government of Canada in collaboration with the other Governments and responsible authorities of the British Commonwealth provides up-to-date information in the form of abstracting and reviewing journals, technical communications and bibliographies on all aspects of science and practice as applied to agriculture, horticulture and forestry.

JOURNALS. The following list comprises journals and other periodical publications. Subscription rates are quoted after each; in certain cases (journal marked with an asterisk) a 20 per cent deduction is made for subscribers in the British Commonwealth who send their subscriptions direct and not through a bookseller.

Bulletin of Entomological Research	(30s.)	Plant Breeding Abstracts*	(25s.)
Review of Applied Entomology (Series A)	(30s.)	Herbage Abstracts*	(25s.)
Review of Applied Entomology (Series B)	(15s.)	Horticultural Abstracts*	(25s.)
Review of Applied Mycology	(30s.)	Animal Breeding Abstracts*	(25s.)
Soils and Fertilizers*	(25s.)	Helminthological Abstracts	(30s.)
Veterinary Bulletin	(40s.)	Dairy Science Abstracts*	(25s.)
Index Veterinarius	(100s.)	Forestry Abstracts*	(25s.)
Nutrition Abstracts and Reviews	(42s.)		

OCCASIONAL PUBLICATIONS, BIBLIOGRAPHIES, ETC.

	PRICE
Some British Books on Agriculture, Forestry and Related Sciences 1939-45	3s.
Revisions of and Additions to Indian Fungi	3s. 9d.
Common Names of Virus Diseases Used in the Review of Applied Mycology	5s.
Land Classification for Land-Use Planning	4s.
The Spectrographic Analysis of Soils and Plants	4s.
Vitamin B_1 in Cereals	2s. 6d.
The New Genetics in the Soviet Union	6s.
The Use of Heterosis in the Production of Agricultural and Horticultural Crops	3s.
The Grasslands of Latin America	7s. 6d.
The Forage Resources of Latin America—Peru	2s. 6d.
Advances in Grassland Husbandry and Fodder Production. 2nd Symp.	4s.
The Production of Seed of Root Crops and Vegetables	3s.
Alternate Husbandry	5s.
Forest Tree Breeding and Genetics	5s.
The Use of Aerial Survey in Forestry and Agriculture	4s.
The Use and Misuse of Shrubs and Trees as Fodder	6s.
A Review of the Literature on Soil Insecticides	10s.
An Introduction to the Taxonomy and Nomenclature of Fungi	5s.
Minerals in Pasture	5s.
Bibliography of Soil Science, Fertilizers and General Agronomy, 1940-44	30s.
The Establishment and Early Management of Sown Pastures	7s.
Spring Frost Damage in Orchards and its Possible Prevention	1s. 6d.
Chemical Composition of Plants as an Index of their Nutritional Status	7s. 6d.
Fruit Fall and its Control by Synthetic Growth Substances	3s. 6d.
Co-operation in Forestry	4s.
Forestry Credit	2s. 6d.
The Application of Meteorology to Forest Fire Protection	2s. 6d.
Management in Selection Forests, with Particular Reference to the Application of the "méthode du contrôle" in Switzerland	3s.

All correspondence regarding above journals and other publications may be addressed in the first instance to:

IMPERIAL AGRICULTURAL BUREAUX,
CENTRAL SALES BRANCH,
(Temporary address) PENGLAIS,
ABERYSTWYTH, WALES.

CANADIAN JOURNAL OF RESEARCH

Notes on the Preparation of Copy

GENERAL:—Manuscripts should be typewritten, double spaced, and the **original and at least one extra** copy submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulae, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. **All pages, whether text, figures, or tables, should be numbered.**

ABSTRACT:—**An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

ILLUSTRATIONS:

(i) *Line Drawings:*—All lines should be of sufficient thickness to reproduce well. Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper **ruled in blue only**; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in **green, yellow, or red should not be used** unless it is desired to have all the co-ordinate lines show. Lettering and numerals should be neatly done in India ink preferably with a stencil (**do not use typewriting**) and be of such size that they will be legible and not less than one millimetre in height when reproduced in a cut three inches wide. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate**; if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—**The author's name, title of paper, and figure number should be written on the back of each illustration.** Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Each figure should be referred to in the text.**

TABLES:—Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Each table should be referred to in the text.**

REFERENCES:—**These should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper.** The form of literature citation should be that used in this Journal. **Titles of papers should not be given in references listed in Sections A, B, E, and F, but must be given in references listed in Sections C and D. All citations should be checked with the original articles.** Each citation should be referred to in the text by means of the key number; in Sections C and D the author's name and the date of publication may be included with the key number if desired.

The *Canadian Journal of Research* conforms in general with the practice outlined in the *Canadian Government Editorial Style Manual*, published by the Department of Public Printing and Stationery, Ottawa.

Reprints

Fifty reprints of each paper are supplied free. Additional reprints, if required, will be supplied according to a prescribed schedule of charges.

VIRUS